Monograph on **Carob Bean Gum** 7-24-72

MONOGRAPH

ON

CAROB BEAN GUM

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CAROB BEAN GUM

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Summary

Kratzer, et al. found that ingestion of carob bean gum causes a reduction in growth rate and feed efficiency. As a 2% constituent of the diet of chicks for a period of 21 days, carob bean gum exhibited a marked depression of the growth rate (6, 11). Ershoff and Wells fed carob bean gum to rats for 28 days as 10% of their diet. A slight growth depression resulted (2). In neither case was the weight depression correlated with toxicity.

Ershoff and Wells reported that carob bean gum counteracts the effects of cholesterol feeding (2).

Holbrook found carob bean gum to be effective in the treatment of constipation. X-ray studies on man reveal that the gum disperses in the large intestine and alters the consistency of the fecal mass, effecting very little increase in stool weight. Carob bean gum does not interfere with digestion in man (4). Rivier reports that clinical studies with infants indicate that 1% carob bean gum has no effect on man (8).

There are no reported allergenic effects of carob bean gum in the scientific literature.

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Bibliography - Summary

2. Ershoff, B. H., and A. F. Wells. 1962. Effects of gum guar, locust bean gum and carrageenan on liver cholesterol of cholesterol-fed rats. Proc. Soc. Exptl. Biol. Med. 110(3):580-582.

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CAROB BEAN GUM

Chemical Information

I. Nomenclature

A. Common Names
1. Carob bean gum
2. Locust bean gum
3. Algaroba
4. St. John's bread gum

B. Chemical Names

Carob bean gum is a high molecular weight hydropolysaccharide composed of D-galactan and D-mannose units combined through glycosidic linkages (1→4). It may thus be described chemically as a galactomannan (10).

C. Trade Names (9)
1. Johannisbrotmehl
2. Arobon
3. Gum Gatto
4. Gum Hevo
5. Jandagum
6. Lakoegum
7. Rubigum
8. Lupogum
9. Luposol
10. Gum Tragon
11. Tragarab
12. Tragasol

D. CAS Registry Number PM9000402

II. Empirical Formula

Carob bean gum is composed of D-galactose and D-mannose units in a galactomannoglycan and has been reported in the following varying ratios of D-galactose to D-mannose (9):

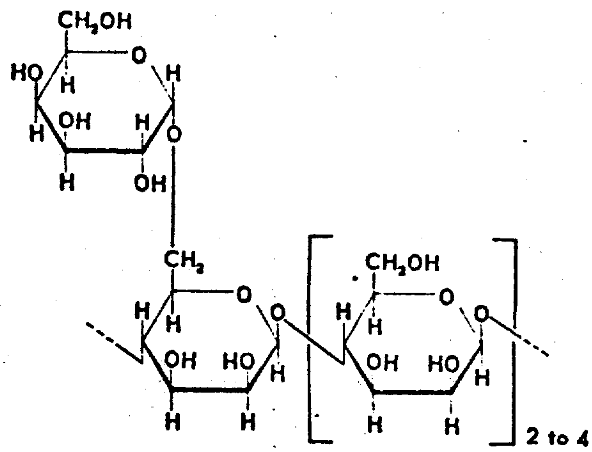
D-galactose	D-mannose
20-14	80-86
17	83
20-25	75-80
27	73

Whistler and Smart point out that these varying ratios may be due not only to variation in analytical methods employed, but also to the origin of the gum or the growth conditions of the plant at the time of production (9).

The carob bean gum molecule is a linear chain of D-mannopyranosyl units linked (1→4) with every fourth or fifth D-mannopyranosyl unit substituted on C6 with a galactopyranosyl unit. Carob bean gum differs from guar gum only in the number of D-galactose side chains (10).

III. Structural Formula

Methylation data has yielded the following structure as the simplest average repeating unit of carob bean gum (10).



Since the specific rotation of the polysaccharide is low, the glycosidic bonds are believed to be mainly of the beta-D- variety (10).

IV. Molecular Weight

Duell and Neukon reported a molecular weight of 310,000 (9).

V. Specifications

A. Chemical (Commercial) (9)

D-galacto-D-mannoglycan	88%
Pentoglycan	4%
Protein	6%
Cellulose	1%
Ash	1%

B. Food (See C)

C. Food Chemicals Codex

Galactomannans	Not less than 73%
Limits of Impurities	
Acid-insoluble matter	Not more than 5%
Arsenic	Not more than 3 ppm
Ash (total)	Not more than 1.2%
Heavy metals	Not more than 20 ppm
Lead	Not more than 10 ppm
Loss on drying	Not more than 15%
Protein	Not more than 8%
Starch	Passes test

VI. Description

A. General Characteristics

Carob bean gum is a white to yellowish white, nearly odorless and tasteless powder. When it is boiled in water it acquires a leguminous taste.

B. Physical Properties

Carob bean gum is incompletely dispersed in water at room temperature. Insoluble flocs are attributed to crystalline regions in the dry gum and disintegrate as the temperature of the dispersion is raised. If the gum is dispersed in cold water, the viscosity decreases as the temperature is raised, followed by a sharp increase. Preheated dispersions that are cooled attain optimum viscosity.

The pH of the dispersion is between 5.4 and 7.0. pH has little effect on the viscosity in the range pH 3-11.

A dispersion of carob bean gum may be gelated by the addition of small amounts of sodium borate (9).

C. Stability

Carob bean gum should be stored in well-closed containers.

VII. Analytical Methods

Carob bean gum solution turns purple when iodine is added and gels when either sodium tetraborate or boric acid is added. It also forms a copper complex with Fehling's solution. Although these tests are not specific for carob bean gum, they are specific for galactomannans. Carob bean gum is precipitated by tannic acid, sulfuric acid and neutral salts such as lead acetate (10).

Ewart and Chapman (3) used the solubility and precipitation properties of carob bean gum to isolate it from mixtures and qualitatively identify it. Their scheme distinguishes between pectin, alginate, gelatin, starch, carboxymethylcellulose, methylcellulose, and several other gums, including carrageenan, tragacanth, agar, karaya, ghatti, and gum arabic, but not guar gum.

Another procedure for the isolation and detection of carob bean gum is to reflux the sample in 50 ml water and 50 ml barium hydroxide in 100 ml of water is added, the pH adjusted to 7, and evaporated to a small volume (1). The solution is then paper chromatographed with butanol:pyridine:water (3:2:1.5) as the mobile solvent and phthalic acid-aniline and naphthoresorcinol-trichloroacetic acid as developing colors. In addition to carob bean gum, this method can be used to separate and identify other gums including gum arabic, tragacanth, several sugars, and pectin.

Padmoyo and Miserez (7) have used microelectrophoresis to separate and identify the gums: arabic, tragacanth, carrageenan, carob and guar, as well as, gelatin, pectin starch, dextrin, agar, sodium alginate, methylcellulose and carboxymethylcellulose. The electrophoresis is carried out on cellulose acetate strips. Substances with similar motility are differentiated by staining.

Carob bean gum can also be qualitatively determined by X-ray diffraction and infrared spectrometry.

VIII. Occurrence

Carob bean gum is obtained from the ground endosperm of the seed of the <u>Ceratonia siliqua</u> (Linne), a leguminous evergreen tree found in the mediterranean area.

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Biological Data

I. Acute Toxicity

No information available

II. Short-Term Studies

Rats

A total of 24 male Holtzmann rats as divided into 3 groups of 8 each and housed 2-3 animals per cage. A control group was fed a stock diet, another group fed stock diet + 1% cholesterol, and a test group fed stock diet + 1% cholesterol + 10% carob bean gum. Test diets were provided ad lib on alternate days, water continually. Body weights were recorded weekly, and the experiment was run for 28 days (2).

Serum cholesterol, hepatic cholesterol, and liver total lipid were determined at the conclusion of the experiment. The increase in hepatic cholesterol and liver total lipid was largely counteracted by feeding 10% carob bean gum. There was a slight depression of body weight in animals fed the carob bean gum, but sufficient data were not included to determine whether this was the result of a depressed food intake or an effect of the gum itself (2).

Chicks

One-day old Arbor-Acres chicks were divided into duplicate groups of 10 each of equivalent weight distribution and housed in wire cage batteries. A control group was fed a stock diet, one test group fed a 2% cellulose supplement, and another group fed a 2% carob bean gum supplement. Feed and water were supplied ad lib. The experiment was run for 3 weeks. Nitrogen retention, fat absorption, and metabolizable energy content were determined (6).

Carob bean gum-fed chicks showed a depressed feed intake and a corresponding depression in weight gain in comparison to cellulose-fed chicks and controls. The degree of nitrogen retention and the metabolizable energy content were about the same as those for cellulose; however, fat absorption was slightly higher (6).

One-day old Arbor-Acres chicks were weighed and separated into groups of 10. A control group was fed a stock diet and five test groups were fed the stock diet modified with carob bean gum in the following proportions: 0.25%, 0.5%, 1%, 2%, and 2%. Groups were provided feed and water ad lib and weighed as a group twice a week. At the end of 20 or 21 days, chicks were weighed individually (11).

Over the 20-21 day period, chicks fed 2% carob bean gum had a growth depression of approximately 27%. The groups fed lower levels of gum showed about 3% depression of growth when compared with control chicks (11).

At the 2% level in the diet, carob bean gum showed a definite depression of the growth rate of chicks; furthermore, this was not the result of the nutritional value of the gum since another non-nutritive supplement, cellulose, in the same concentration did not show this effect. This effect, however, cannot, from the data given, be attributed to a toxic nature of carob bean gum. No data were provided on food intake of the test chicks as compared to that of the controls (11).

In summary, the growth of chicks was markedly affected by 2% carob bean gum; whereas, the growth of rats was not significantly affected by 10% carob bean gum.

Man

Several different studies examining the laxative action of carob bean gum were carried out. Eight individuals were fed barium suspensions followed by 2 heaping teaspoonsful of carob bean gum. X-ray studies followed. Another similar study involving 9 individuals used a carob bean gum-barium suspension mixed before ingestion. In another variation, this barium-carob bean gum suspension was given to 10 volunteers, and X-ray studies were performed 1, 3, 8, and 24 hours after ingestion. Other comparative studies involved psyllium seed gum and Sterculia (4).

X-ray studies and stool examinations showed that carob bean gum permeates the fecal mass in the colon and mixes with it. Its greatest effect is the alteration of consistency resulting in little actual increase in stool weight. Carob bean gum does not disperse as a gel until it reaches the large intestine; whereas, the other examined gums do. There is, as a result, no interference with digestion in man (4).

In a series of clinical experiments on infants, Rivier found that the duration of gastrointestinal transit was unchanged by 1% carob bean gum. Furthermore, of the 16 infants fed carob bean gum for an unstated period of time, there were no untoward effects reported (8).

III. Long-Term Studies

None

IV. Special Studies

None

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Biochemical Aspects

I. Breakdown

No information available from sources obtained.

II. Absorption - Distribution

No information available from sources obtained.

III. Metabolism and Excretion

No information available from sources obtained.

IV. Effects on Enzymes and Other Biochemical Parameters

When day-old Arbor-Acres chicks were given 2% carob bean gum in their diet for 21 days, they showed a significant growth depression (40%) and a reduction of feed efficiency. At a marginally low level of dietary vitamin D_3, a reduction in the bone ash was observed. This was due, possibly, to poor absorption of D_3, thereby creating a deficiency state resulting in poor bone mineralization (6).

The increment in liver cholesterol and liver total lipid induced by cholesterol feeding (1% for 28 days) in the rat was largely counteracted by concurrent feeding of carob bean gum (10%) (2).

Small pellets of carob bean gum are used as a treatment for constipation. Upon ingestion by man they pass through the stomach and small intestine, into the large intestine, before disintegration. The maximal colloidal mass of the gum is reached in the colon, aiding in relief of constipation by causing an increase in bulk. No adverse effects caused by the gum were observed in the 19 subjects studied (4).

V. Drug Interaction

No information available from sources obtained.

VI. Consumer Exposure Information

The major uses of carob bean gum in the food industry include ice cream, soft-cheeses, cheese spreads, ground meat products, canned meat and fish, sauces, salad dressing, pie fillings, and bread dough (5, 9).

Carob bean gum is listed as GRAS for use as a stabilizer (5).

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IDENTIFICATION BY PAPER CHROMATOGRAPHY OF SWELLING AGENTS IN FOODSTUFFS

Testing for swelling agents or thickeners in pure form presents no difficulties. A compilation by Letzig (1) lists a number of precipitating reactions which permit distinguishing between the several thickeners in simple manner. However, these compounds seldom exist in such pure form that the use of such precipitating reactions, presented in synoptic form, permits non-ambiguous conclusions. Even the occurrence of two different swelling agents simultaneously can make a clear identification difficult; when there are more than two, this is true even more so. However, not only a mixture of these substances complicates the tests but also the presence of other compounds among which the proteins are predominant. Letzig's reagents are mainly protein precipitants so that precipitation will always occur in the presence of proteins. It is easy, of course, to determine the presence of proteins but this does not yet allow us to decide whether, in the absence of proteins, precipitation will have been due to thickeners. Prior to testing, proteins must therefore be removed and Wyler (2) has already described a possibility for this, specifically for meat products. This suggested removal of proteins with the aid of Carrez precipitation is so thorough, however, that not only are many swelling agents precipitated for which we must thus test prior to precipitation but the possibility of identifyin those still remaining in solution is also restricted to a very few. A method of complete removal of proteins without interfering with subsequent testing and identification of swelling agents is not yet known so that the Letzig reagents in practice unfortunately have only a limited importance. As seductive as such tests are in their simplicity, they alone very rarely furnish non-ambiguous indications of the kind of the swelling agents present. There is a possibility of precipitating the proteins with alcohol which can subsequently be easily evaporated (e.g. in vacuum) but this has the disadvantage that the proteins are not always completely eliminated and that some thickeners, specifically carob-seed flour, are also precipitated.

These disadvantages raise the question of different testing methods and Letzig already indicates in the report cited that the application of paper chromatography promises success. Since most thickeners are constituted by carbohydrates, no difficulties exist from the point of view of paper chromatography; it is merely necessary to convert the test substance into a form suitable for paper-chromatographic testing. It may happen, however, that with several swelling agents present, a non-ambiguous conclusion on the kind of the individual substances is not possible since a number of thickeners have a similar composition and/or furnish similar cleavage products after hydrolysis. In that case, however, a combination with the precipitating reagents of Letzig would permit us to continue further since the decision will lie between the few still possible thickeners. Whereas the proteins do not interfere with such paper-chromatographic tests, we must first test for the presence of carbohydrates not originating from swelling agents. Admixtures of simple sugars easily determinted with the aid of paper chromatography, can generally be removed by extracation with 50-% alcohol which does not affect the swelling agents. It is sufficient to let the substance stand with the alcohol for several hours under occasional shaking. The extract and/or the filtrate can serve to test for sugar (3) whereas the thickeners can be determined from the residue.

Hydrolysis of Swelling Agents

Since thickeners do not directly respond to paper chromatography, it is necessary to convert them to a form which is accessible to this method of testing. Among the possibilities of hydrolytic cleavage, that using enzymes is excluded and, since carbohydrates are involved, acid hydrolysis is indicated primarily in order to prevent decomposition of cleavage products. The following conditions have been shown to be suitable and have been steadily employed for some time:

10 g of pure swelling agent and/or test substance are diluted with 50 ml of water and 50 ml of a 10-% sulphuric acid solution. If the mixture is too thick so that the retort may burst under direct heating, it should be placed in a hot water bath for a short time. When fluid, the solution is boiled in a reflux condenser where duration is a function of the kind of the probable thickeners since each substance is hydrolyzed at different rates of speed. If no indication of the type of swelling agent exists, the solution is kept at boiling temperature for three hours. It can then be directly chromatographed. However, it is preferable to concentrate if there is only a small amount of test substance; sulphuric acid also interferes somewhat, specifically with uronic acids, so that it is preferable to remove the former. For this purpose, 30 g barium hydroxide (with 8 mol water) are dissolved in 100 ml of water under heating and dripped, under constant shaking, through a filter into the hot hydrolytic solution until the latter reaches a p_H of 7. With these generally known conditions for removal of the sulphuric acid, it is easy to adjust the p_H-value as above by spotting Merck indicator paper. The precipitated barium sulphate can be rapidly filtered out, after settling, through a standard filter. Since only small amounts are required, it is sufficient if a part of the filtrate is processed further. This part is concentrated under vacuum to 50% and thus reaches again the starting concentration. If only small amounts of swelling agent are available, these can be further concentrated as desired. This solution is now suitable for paper chromatography. It is not at all necessary to keep within the quantitative relations indicated above and they can all be reduced correspondingly which is of value when only small amounts of test substance are available.

Paper-Chromatographic Conditions

Paper chromatography always takes place under the conditions already described earlier (3 & 4). The fluxing agent is n-butanol/pyridine/water (3:2:1.5) and staining is done with phtaleic acid/aniline and/or naphthoresorcinol/trichloroacetic acid. The last-named staining for non-reducing sugars plays a subordinate role in this connection. The chromatograms thus obtained with the ascending method are shown in the figure at the end of the article. The parallel obtained test solutions permit non-ambiguous identification so that even closely adjacent spots cannot be confused with each other which may easily occur when working exclusively with R_f-values.

Evaluation

Fig. 1 shows two chromatograms of some frequently occurring swelling agents. It will be clearly seen that, after hydrolysis of carob-seed flour, only the spots of galactose and mannose appear. Although mannose is nearly equal in R_f-value to mannose, it can be distinguished from the reddish arabinose by its brown color tone but these colors cannot be recognized in a black-and-white photo. With agar-agar, only galactose is clearly seen whereas xylose is manifested only as a weak spot. Gum arabic shows galactose in addition to arabinose and rhamnose. Although rhamnose is recognized only vaguely, it is characteristic since it is only infrequently found in thickeners. In the sample examined by us, there occurs further a spot immediately below the starting point which we were never able to identify. Pectin shows mainly the spot of uronic acid which can be recognized from the typical light-brown color after staining with phthalate/aniline as well as by staining with naphthoresorcinol/trichloroacetate when it turns slightly blue after some time (cf. 3 for details). We know that this involves galacturonic acid which paper chromatography cannot demonstrate since sufficient separation from glucuronic acid does not take place. The R_f-values of the two uronic acids do differ slightly and we might therefore assume that differentiation would be possible after sufficient time. Here we must take into account that the R_f-values of the two acids are to a considerable extent a function of the concentration of these acids and of the kind of an eventually existing cation. Something similar has already been shown for some other organic acids (5). The variations so caused are greater, however, than the difference between the two R_f-values. In addition to the spot of galacturonic acid, a weak spot of glucose also occurs with pectin. It must be remembered further that a duration of hydrolysis of three hours must be strictly kept, in contrast to most other swelling agents. Whereas a duration of hydrolysis of one to two hours generally produces complete cleavage in the latter, galacturonic acid in pectin shows only weakly after one hour but increases with continued hydrolysis. Tragacanth gum shows the spots of galactose, arabinose and xylose. Comparison with the test solutions which were run parallel on the left chromatogram, makes possible rapid identification of the individual spots. The first test solution on the left shows the upper spot as galactose and the lower spot as mannose; the second test solution indicates the upper spot as arabinose and the lower as rhamnose. We already mentioned that arabinose and mannose cannot be differentiated in the figure since it is not possible to see the differences in color. The chromatogram makes it impossible to mix up the red color of arabinose with the brown color of mannose. The last test solution shows galacturonic acid at the top and, below this, xylose.

The chromatogram on the right of the figure shows "tylose" (methyl cellulose) on the left side which shows as expected the spot of glucose and below this non-hydrolyzed methylated glucoses. The occurrence of these methylated glucoses was in several preparations and appears to be characteristic for most of the tylose preparations. It is possible, however, that this is a function of manufacture and that these spots may therefore be absent some times. The adjacent cellulose glycolate also shows the spot of glucose and, in the upper part of the chromatogram, two and occasionally three unidentified spots which always have a characteristic semilunar form. Alginate exhibits only the spot of a uronic acid. The adjacent test solutions show in the first case from top to bottom the spots of lactose, maltose and glucose whereas glucuronic acid is shown on the left.

When several thickeners are present, identification on the basis of the chromatogram can become difficult. It then seems preferable to make identification less through the spots that do show but initially through those absent. The absence of uronic acid permits a definite conclusion that neither pectin nor alginate exist. However, we believe sufficient concentration of the hydrolyzed solution must be made in order to avoid that a uronic acid does not show by reason of excessive dilution. The absence of galactose excludes the presence of carob-seed flour, agar-agar, gum arabic and tragacanth. Absence of glucose means that neither tylose nor cellulose glycolate, generally also no pectin and obviously no swelling starch can be present. Lack of mannose would exclude carob-seed flour and that of arabinose would eliminate gum arabic and tragacanth. Here we must take into account, however, that the presence of one of the two last-named spots does not permit a conclusion on the absence of the other since a possible superposition may exist which does not allow this.

With this method of selection, most of the known swelling agents can easily be excluded so that we do obtain in general a non-ambiguous picture of the composition of the mixture of swelling agents. Where in complicated cases several interpretations are possible, we can turn to the precipitating reactions Letzig mentioned above for clarification of any remaining doubts, in spite of the interferences indicated. When direct interpretation is not possible, the method of selection has proved itself also for the Letzig reactions. In most cases, however, paper chromatography should be sufficient or at least give decisive indications. As in most investigative methods, a certain intuitive judgment will develop with practice.

As described, such testing for swelling agents is obviously restricted only to those whose composition includes carbohydrates. Although these represent the greater number, this does not allow us to neglect others, e.g., gelatine. Testing for such substances by specific paper chromatography would evidently have to be carried out from other viewpoints.

Conclusion

1. The precipitating reactions in testing for swelling agents are excellent for pure compounds but rarely furnish non-ambiguous findings for mixtures and in the presence of other substances, e.g. proteins.

2. Since most of the swelling agents are constituted by carbohydrates, paper chromatography of the sugars and/or sucroid compounds offers the possibility of identifying swelling agents by hydrolytic cleavage.

3. Hydrolysis is made with sulphuric acid and boiling at an average duration of 3 hours. After removal of sulphuric acid by barium hydroxide and concentration of the clear filtrate under vacuum, the test is made with a method described in an earlier communication.

4. Evaluation is best made by a method of selection in which all swelling agents are excluded which cannot exist due to the absence of a spot. However, it is necessary to take into account the ratios of concentration.

3)

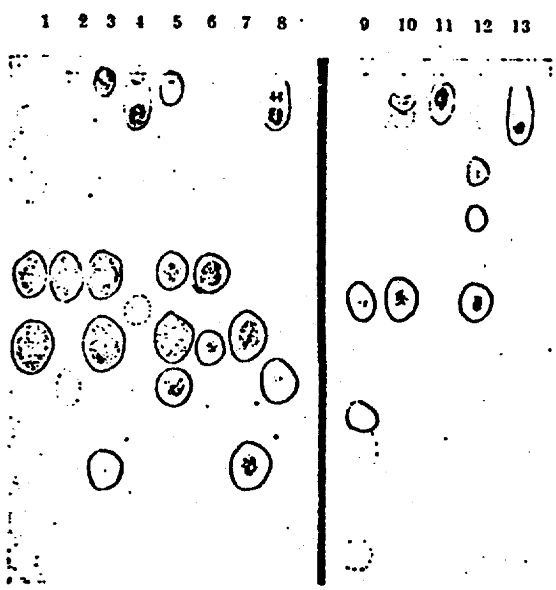

Abb. 1. *Mit Schwefelsäure hydrolysierte Quellstoffe und entsprechende Testlösungen.* Fließmittel: n-Butanol/Pyridin/Wasser (3:2:1,5). Färbung: Phthalsäure/Anilin. *Linkes Chromatogramm:* (von links) *1* Johannisbrotkernmehl, *2* Agar-Agar, *3* Gummi-arabicum, *4* Pektin, *5* Traganth. Testlösungen: *6* Galaktose + Mannose, *7* Arabinose + Rhamnose, *8* Galakturonsäure + Xylose. *Rechtes Chromatogramm:* (von links) *9* Tylose, *10* Celluloseglykolat, *11* Alginat. Testlösungen: *12* Lactose + Maltose + Glucose, *13* Glucuronsäure

Fig. 1 - Chromatogram of swelling agents hydrolyzed with sulphuric acid and the resulting test solutions. Fluxing agent: n-butanol/pyridine/water (3:2:1.5). Staining: Phthaleic acid/aniline. Left side: 1= carob-seed flour; 2 = agar-agar; 3 = gum arabic;

4 = pectin; 5 = tragacanth; test solutions: 6 = galactose + mannose; 7 = arabinose + rhamnose; 8 = galacturonic acid + xylose. Right side: 9 = tylose; 10 = cellulose glycolate; 11 = alginate; test solutions: 12 = lactose + maltose + glucose; 13 = glucuronic acid.

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Effects of Gum Guar, Locust Bean Gum and Carrageenan on Liver Cholesterol of Cholesterol-Fed Rats.* (27585)

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Our previous studies(1) indicate that pectin N.F. when fed at a 5% level in the diet largely counteracted the increment in liver cholesterol and liver total lipids induced by cholesterol feeding in the rat. Other roughage or bulk-forming materials such as cellulose, agar, sodium alginate, protopectin and calcium silicate (Micro-Cel) were ineffective in this regard. Data are presented here indicating that Gum Guar, Locust Bean Gum and carrageenan also have significant activity in counteracting the increment in liver cholesterol and liver total lipids induced by cholesterol feeding in the rat.

Procedure. The basal ration consisted of sucrose, 61%; casein,† 24%; cottonseed oil, 10%; salt mixture,‡ 5%; and the following vitamins per kg of diet: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; p-aminobenzoic acid, 400 mg; inositol, 800 mg; 2-methyl-1,4 naphthoquinone, 5 mg; vit B_{12}, 150 μg; choline chloride, 2 g; vit A, 5000 U.S.P. units; vit D_2, 500 U.S.P. units; and alpha-tocopheryl acetate, 100 mg. Tests were conducted with rats fed the basal ration, the basal ration + 1% cholesterol, and the basal ration + 1% cholesterol + the various supplements indicated in Table I. The cholesterol and test supplements were incorporated in the basal ration in place of an equal amount of sucrose. Fifty-six male rats of the Holtzman strain with an average body wt of 43.6 g (range 38 to 50 g) were divided into 7 comparable groups of 8 each, placed in metal cages with raised screen bottoms (2 or 3 rats per cage), and provided the test diets and water *ad libitum*. Animals were fed on alternate days and all food not consumed 48 hours after feeding was discarded. Body weights were recorded weekly. After 28 days of feeding, the rats were anesthetized with sodium pentobarbital, and blood was withdrawn from the aorta into a heparinized syringe. Livers were excised, blotted to remove excess blood, weighed and stored in a freezer until analyzed. Lipid was extracted from the livers by the method of Thompson *et al.*(2), and cholesterol was determined on liver and plasma by the method of Nieft and Deuel(3). Total lipids were determined gravimetrically on an aliquot of the liver extract.

Results. The increment in liver cholesterol and liver total lipid induced by cholesterol feeding in the rat was largely counteracted by the concurrent feeding of Gum Guar, Locust Bean Gum or carrageenan at a 10% level in the diet. The effects of these supplements were similar to, although slightly less marked than, that of a comparable amount of pectin N.F. Cholesterol feeding also induced a slight increment in plasma cholesterol levels which was partially counteracted by concurrent feeding of pectin N.F. and several of the other test supplements. Differences between the various groups however were not statis-

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† Vitamin-Free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Hubbell, Mendel and Wakeman Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

TABLE I. Effects of Dietary Supplements on Plasma and Liver Cholesterol and Liver Total Lipid of Cholesterol-Fed Rats (8 Animals/Group).*

Supplements fed with basal ration	Body wt at sacrifice, g†	Plasma cholesterol, mg/100 ml†		Liver cholesterol, mg/g†		Liver total lipid, %†
		Free	Total	Free	Total	
None	197.4 ± 4.9	20.1 ± 1.6	85.2 ± 5.8	1.63 ± .09	2.09 ± .09	4.74 ± .3
1% cholesterol	201.3 ± 6.8	21.0 ± 1.1	105.5 ± 5.0	2.72 ± .14	16.17 ± 1.42	9.03 ± .7
Idem + following supplements:						
5% pectin N.F.	185.4 ± 6.1	19.6 ± 1.0	92.8 ± 4.2	2.45 ± .13	5.59 ± .64	6.31 ± .4
10% " "	181.7 ± 5.6	18.2 ± 1.4	95.6 ± 3.3	2.38 ± .17	4.12 ± .42	4.78 ± .3
10% Gum Guar	177.6 ± 5.2	20.8 ± 1.2	91.3 ± 4.3	2.29 ± .12	6.05 ± .79	6.29 ± .5
10% Locust Bean Gum	180.3 ± 5.4	24.0 ± 1.5	103.3 ± 5.0	2.38 ± .08	7.59 ± .73	6.81 ± .4
10% carrageenan	191.9 ± 3.8	20.8 ± 1.6	89.3 ± 5.5	2.22 ± .09	5.52 ± .62	5.86 ± .3

* Test supplements obtained from the following sources: pectin N.F. (citrus), Sunkist Growers, Ontario, Calif.; Gum Guar and Locust Bean Gum, Hathaway Allied Products, Los Angeles, Calif.; carrageenan (Gelcarin MR 100), Marine Collids, Inc., of America, New York. Test supplements were all natural sources of hydrophyllic complex carbohydrate colloids made up of repeating units of (1) galacturonic acid in the case of pectin, (2) mannose and galactose in Gum Guar and Locust Bean Gum, and (3) sulphated galactose in Gelcarin MR 100. The pectin N.F. employed was a purified material obtained from the dilute acid extract of the inner portion of the rind of citrus fruits. It had a methoxyl value of 10.7% on a moisture-ash-free basis. Gum Guar was the ground endosperm of Guar (known botanically as *Cyamopsis tetragonolaba*) seed with a galactomannan content of approximately 80%. Locust Bean Gum was obtained from the endosperm of the kernels of the Carob tree. It was a hemicellulose product of about 4 parts mannose and 1 part galactose. Gelcarin MR 100, a highly purified product designed for use in milk or milk products, is known chemically as carrageenan. The latter occurs naturally in a number of red seaweeds (class Rhodophyceae) but is obtained principally from the group of seaweeds known as Irish moss.

† Including stand. error of mean.

tically significant.§ Findings are summarized in Table I.

Discussion. A number of studies indicate that, in general, populations habitually subsisting on diets low in fats and animal protein tend to have a low concentration of serum cholesterol and a low incidence of cardiovascular disease(4-7). Such diets contain a number of constituents which are either absent from or present in only minute amounts in the diets of populations with a high incidence of hypercholesterolemia, atherosclerosis and coronary artery disease. Walker and Arvidsson(8) and Higginson and Pepler(9) were among the first to call attention to the high fiber content of the Bantu diet as a possible explanation for the low serum cholesterol level observed in the Bantu population. A similar suggestion was also made by Bersohn *et al.*(10). More recently Keys *et al.*(11) conducted controlled experiments in which groups of physically healthy men subsisted alternately on "American" and "Italian" types of diets, devised to be comparable in calories, proteins and in kind and amounts of fat but differing in the sources of carbohydrates. An abundance of fruits and vegetables in the Italian type diets (which tended to be high in complex carbohydrates such as pectins, hemicelluloses and fiber) replaced equivalent calories in simpler carbohydrates in the American type. Serum cholesterol lev-

§ Food intake was determined for rats in the various groups. Since the weight increment of rats fed the test supplements was less than that of rats fed the basal + cholesterol diet, the question arose whether the reduction in weight increment of rats fed the test supplements may not have been due to a reduction in amount of diet and hence cholesterol ingested and whether the reduced cholesterol intake in turn may not have been the cause of lower liver cholesterol and liver total lipid levels. Such, however, does not appear to be the case, for differences in body weight between the various groups were not statistically significant whereas differences in liver cholesterol and liver total lipid were. Furthermore, an even greater reduction in body weight was observed (unpublished findings) in rats fed the basal ration + cholesterol + 20% alfalfa meal without an accompanying reduction in liver cholesterol and liver total lipid values. The possibility that the test supplements inhibited absorption of cholesterol from the gut, however, has not been excluded.

els were significantly lower with the "Italian" type diets. In subsequent studies Keys *et al.* (12) reported that citrus pectin when fed at a level of 15 g per day caused a slight but statistically significant reduction in serum cholesterol levels in physically healthy, middle-aged men; cellulose (fiber) fed under comparable conditions was without significant effect. These studies suggest that cellulose or fiber *per se* was not responsible for the low serum cholesterol levels of the Bantu and comparable groups, but that pectin, another complex bulk-forming carbohydrate, may have had some activity in this regard.‖ An analysis of the diets of native populations with low serum cholesterol levels indicated that in addition to pectin such rations also contain gums and/or other complex carbohydrates such as colloids of marine plants which were found in the present experiment to cause a highly significant reduction in liver cholesterol levels in the cholesterol-fed rat. Further studies are indicated to determine what effect these substances might have, when administered alone or in combination with one another and pectin, in treatment of hypercholesterolemia and atherosclerosis in man.

‖ Unpublished studies from this laboratory indicate that different batches of pectin may vary markedly in anti-cholesterol activity. Pectic preparations with a methoxyl content of 5% or less were without activity in counteracting the increment in plasma and liver cholesterol levels induced by cholesterol feeding in the rat in contrast to the marked activity exhibited by pectin N.F. preparations of relatively high methoxyl content (10.7% on a moisture-ash-free basis).

Summary. The increment in liver cholesterol and liver total lipid induced by cholesterol feeding in the rat was largely counteracted by concurrent feeding of Gum Guar, Locust Bean Gum or carrageenan at a 10% level in the diet. Effects were similar to, although slightly less marked, than that obtained with a comparable amount of pectin N.F.

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Identification of Stabilizing Agents

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EXTENSIVE use of a variety of polysaccharides as stabilizing or thickening agents in foods has created a need for analytical methods for the identification of commercially available materials and for their detection and quantitative estimation in food products. A number of stabilizers, which are used or have been suggested for use in foods, are listed and their chemical nature is indicated in Table I. Throughout this report the term "gums" is used in referring to all of these materials. Gelatin is included because it has many uses similar to those of the polysaccharides.

As the initial step in a project concerned with the development of the required analytical procedures, methods for the qualitative identification of a number of gums have been studied. Several procedures for the identification of gums are found in the literature. A method described by Jacobs and Jaffe (*15*) classifies the gums on the basis of physical characteristics or appearance of precipitates and thus requires considerable experience on the part of the analyst. Their outline does not include pectic substances, alginates, methylcellulose, carboxymethylcellulose, gum ghatti, starch, or gelatin. It also has the disadvantage of using an unstable reagent (Millon's) which must be freshly prepared each day. A method developed by Cannon and as-

The present work was undertaken to develop a method for the identification of stabilizing and thickening agents used in food products. The materials studied were pectin, de-esterified pectin, algin, Irish moss, gum tragacanth, gum karaya, locust bean gum, starch, agar, gum arabic, gum ghatti, carboxymethylcellulose, methylcellulose, and gelatin. A proposed identification scheme is based on precipitation reactions with calcium chloride, sodium hydroxide, barium hydroxide, and lead acetate. In addition, reactions of the stabilizing agents with a cationic soap, ammonium sulfate, mercuric nitrate, papain, and gelatin are listed. The proposed scheme should be useful for identification of unknown stabilizing agents. A number of the reactions reported might be employed for the identification of individual stabilizing agents in mixtures of these materials or isolated from foods.

the Association of Official Agricultural Chemists (*3*, *5*) does not provide for the identification of pectic substances, alginates, methylcellulose, carboxymethylcellulose, locust bean (carob) gum, or gum ghatti. Bryant (*4*) has described a procedure for distinguishing between pectin and certain gums, but it does not provide for positive identification of the gums. A number of other publications dealing with characteristic properties of these polysaccharides have been summarized by Mantell (*16*), but a systematic procedure is still needed for their identification. Such a procedure would be useful for the identification of products used as thickeners or stabilizers in foods, drugs, and cosmetics and ultimately for the identification of polysaccharides isolated from these materials.

In the present investigation, the manner in which the gums disperse in water after being wetted with alcohol has been a valuable index to the identity of unknown samples. Their solubility properties are summarized in Table II. Use has also been made of the fact that many of the polysaccharides occur as salts of complex organic acids (Table I). The acidic properties may be due to the presence of uronic acid groups, as in gum arabic, or to the unesterified portion of sulfuric acid molecules esterified with the polysaccharide. When mineral acids are added to aqueous solutions or dispersions of these salts, the effective concentrations of the polysaccharide anions are decreased. Thus, although the complex anions may yield insoluble salts with heavy metal cations, most are not precipitated from acid solutions. The amount the pH must be raised in order to precipitate the heavy metal salts—e.g., barium, mercury, or lead—is frequently characteristic of the individual polysaccharide.

Table I. Source and Chemical Nature of Materials Commonly Used as Thickening Agents in Foods

Material	Source	Principal Components	References
Pectic substances	Fruits	Galacturonic acid (occurs as methyl ester)	(*18*)
Algin (sodium alginate)	Seaweeds	Mannuronic acid (Na salt)	(*16*)
Irish moss	Seaweeds	Galactose, galactose 4-sulfate (K and Ca salts)	(*18*)
Agar	Seaweeds	Galactose (D- and L-), galactose 6-sulfate (Ca and Mg salts)	(*18*)
Tragacanth	Plant gum	L-Fucose, D-xylose, galacturonic acid, L-arabinose, D-galactose	(*18*)
Methylcellulose	Modified cellulose	Methyl-D-glucose	(*18*)
Starch	Plants	D-Glucose	(*18*)
Carboxymethylcellulose	Modified cellulose	Carboxymethyl-D-glucose	(*16*)
Locust bean gum (carob gum)	Seed endosperm	Mannose and galactose	(*16*)
Guar gum	Seed endosperm	Mannose and galactose	(*20*)
Karaya	Plant gum	Galactose, acetic acid, galacturonic acid, rhamnose, tagatose	(*14*)
Arabic (acacia)	Plant gum	D-Glucuronic acid, D-galactose, L-arabinose, rhamnose (mixed Ca, Mg, and K salts)	(*18*)
Ghatti	Plant gum	L-Arabinose, galactose, galacturonic acid (Ca salt)	(*16*)
Gelatin	Modified protein	Amino acids	

EXPERIMENTAL

During the present investigation 0.5 to 1.0% aqueous dispersions of the polysaccharides were used for the tests. Aliquots of from 3 to 5 ml. were treated with varying concentrations of the reagents which it was hoped would give characteristic precipitation reactions. Initially the reagents used were those for which Jacobs and Jaffe (*15*) have described reactions with several polysaccharides. Subsequently a number of other reagents were used.

Table II. Dispersion in Water of Gums, Wetted with Alcohol

Gum	Manner of Dispersal in Water
Pectic acid	Insoluble
Pectate (Na, K, or NH_4 salts)	Forms either clear or turbid solution on heating
Pectate (Ca salts)	Insoluble
Pectin	Swells in cold water and dissolves on heating
Alginate	Dissolves slowly in cold water or quickly on heating to form viscous solution
Irish moss	Dissolves slowly in cold water, rapidly on heating to form viscous solution
Agar	Swells in cold water, dissolves on heating, gels on cooling
Tragacanth	Swells to form viscous dispersion in cold or hot water, but does not form true solution
Methylcellulose	Dissolves slowly in cold water but becomes cloudy or gels on heating
Starch	Disperses on heating
Carboxymethylcellulose	Dissolves slowly in cold water, rapidly on heating, giving clear viscous solution with some fine fibrous suspended material
Locust (carob)	Forms viscous suspension but not a true solution
Karaya	Forms viscous suspension. Insoluble particles settle on standing
Arabic (acacia)	Dissolves in cold water to form a clear only slightly viscous solution
Ghatti	Dissolves to form almost clear solution but some insoluble material may remain as fine suspension
Gelatin	Swells in cold water and dissolves on heating

Reactions which were found useful for characterizing the gums are summarized in Tables III and IV. Only those materials having anionic components, such as alginates, or potential anionic components, such as pectin, give pronounced reactions with cationic soap (Table III). As in the case of precipitates with heavy metals, the precipitates with the cationic soap quickly disperse on acidification of the medium. Ammonium sulfate is of interest, in that it gives pronounced precipitation tests with several of the gums but not with alginates, pectin, tragacanth, karaya, arabic, or ghatti, each of which probably contains uronic acid components. The reactions with Stokes's acid mercuric nitrate illustrate the effects of low pH on precipitation of heavy metal salts of the polysaccharide acids. An excess of the reagent makes the solutions strongly acidic and thus the weakly dissociated acids redisperse. Alginic acid and pectic acid are insoluble and thus are not dissolved by excess Stokes reagent. Papain and gelatin give pronounced precipitation reactions only with those gums having anionic components. These precipitates are found only if the

Table III. Precipitation Reactions of Polysaccharide Gums and Gelatin

Gum	1 Vol. 1% Solution of Cationic Soap[a]	0.5 Vol. Saturated Ammonium Sulfate	Diluted[b] Stokes's Acid Mercuric Nitrate Added Dropwise	1 Vol. 2% Papain (6)[c]	1 Vol. 2% Gelatin[c]	4 Vol. 95% C_2H_5OH + 2 – 3 Drops Saturated NaCl
De-esterified pectin	Fine opaque precipitate	Gelatinous translucent precipitate	Gels (almost opaque). Insoluble in excess reagent	Precipitate	Precipitate	Gelatinous precipitate, gels (1 vol.)
Alginate	Fine opaque precipitate	Nil	Gels (almost opaque). Insoluble in excess reagent	Precipitate	Precipitate	Gelatinous precipitate (1 vol.) becomes stringy with 4 vol. alcohol
Pectin	Flocculent precipitate	Nil	Forms almost opaque gel which dissolves in excess reagent	Cloudy	No definite effect	Transparent gelatinous precipitate. Gels (1 vol.)
Irish moss	Stringy or flocculent precipitate	Gelatinous precipitate or gel	Transparent gel. Redispersed by excess reagent	Precipitate	Precipitate	Stringy precipitate
Agar	Gelatinous precipitate	Flocculent precipitate	Turbid or cloudy	Cloudy	Precipitate	Fine flocculent precipitate
Tragacanth	Fine opaque precipitate	Nil	Flocculent precipitate. Dissolves in excess reagent	Precipitate	Precipitate	Voluminous precipitate, jellylike
Methylcellulose	Nil	Precipitate	Nil	Nil	Nil	Nil
Starch	Nil	Precipitate	Nil	Nil	Nil	Opaque flocculent precipitate
Carboxymethylcellulose	Gelatinous clotted precipitate	Gelatinous precipitate	Precipitate dissolves in excess reagent	Precipitate	Precipitate	Voluminous clotted precipitate
Locust	Nil	Precipitate (voluminous)	No pronounced effect	No pronounced effect	Nil	Voluminous opaque stringy precipitate, forms clot
Karaya	Flocculent precipitate	Nil	Flocculent precipitate dissolves in excess reagent	Precipitate	Precipitate	Flocculent precipitate, discrete particles
Arabic (acacia)	Precipitate (very fine)	Nil	Flocculent precipitate dissolves in excess reagent	Precipitate	Precipitate	Fine opaque nonsettling precipitate
Ghatti	Fine precipitate	Nil	Nil	Nil	Precipitate	Fine precipitate, nonsettling (2–3 vol.)
Gelatin	Precipitate in alkaline medium (22)	Precipitate	Nil	Nil	Nil	Finely flocculent precipitate, coagulates

[a] Rodalon (alkyl dimethyl benzyl ammonium chloride), Fairfield Laboratories, Plainfield, N. J.
[b] Mercury dissolved in twice its weight of concentrated nitric acid and diluted to 100 times its volume with distilled water.
[c] Precipitates with papain and gelatin are observed only in weakly acidic medium and most exhibit properties of coacervates rather than true precipitates.

pH of the mixture is below the isoelectric point of the protein and it is possible that they would be more correctly called coacervates. They are usually dispersed by a few drops of mineral acid or of dilute ammonium hydroxide. The characteristic manner in which some of the gums are precipitated by alcohol may also be of value in their identification.

The reactions described in Table IV form the basis of a proposed procedure for the systematic identification of the gums.

REAGENTS

Calcium chloride ($CaCl_2$), 3% solution (weight/volume).
Ammonium hydroxide, 3.0 N solution.
Sodium hydroxide, 3.0 N solution.
Barium hydroxide, saturated solution stored in a bottle equipped with a siphon and a soda-lime tube.
Basic lead acetate, 20% suspension (weight/volume). Heat to boiling, cool, and use supernatant solution.
Hydrochloric acid (or other mineral acid), 3.0 N solution.
Methylene blue, 0.1% aqueous solution.
Tincture of iodine (U.S.P., *14*).
Iodine–potassium iodide stock solution, containing 0.5% iodine and 1.0% potassium iodide. Iodine–potassium iodide test solution, consisting of stock solution diluted 1 to 5.
Cupric sulfate ($CuSO_4.5H_2O$), 15% solution (weight/volume).
Borax ($Na_2B_4O_7.10H_2O$), 4% solution.
Ruthenium red test solution (*3*).
Picric acid, saturated aqueous solution.

IDENTIFICATION PROCEDURE

Wet a 0.25- to 0.5-gram sample of the material to be identified with 1 to 2 ml. of 95% alcohol and add 50 ml. of distilled water. Suspend the solid material in the water by shaking or stirring. Heat the suspension, with frequent shaking, on a hot plate or over a burner. If the sample dissolves, discontinue heating immediately; otherwise hold at 85° to 95° C. for 15 minutes.

Group A. I. Treat a 3- to 5-ml. aliquot of the solution with 0.2 volume of 0.25 M calcium chloride. A gelatinous precipitate or gel indicates alginates or de-esterified pectin.

If no reaction is apparent with calcium chloride alone, add 1 volume of 3 N ammonium hydroxide to the calcium chloride treated sample. Slow formation of a gel or gelatinous precipitate indicates pectin.

II. If either test in A I was positive, mix a fresh 3- to 5-ml. aliquot of sample with 1 volume of 3.0 N sodium hydroxide. Observe the reaction and then heat the mixture in a boiling water bath for 10 minutes.

Immediate formation, in the cold, of a gelatinous or flocculent precipitate indicates either pectin or de-esterified pectin. No precipitate indicates alginates. All three mixtures become yellow on heating, but the precipitates with pectic substances do not dissolve.

Group B. If the material is not an alginate or a pectic substance, carry out the following tests.

I. Mix a 3- to 5- ml. aliquot of the sample with 0.1 volume of saturated barium hydroxide. Observe in the cold and heat in boiling water bath for 10 minutes.

Formation in the cold of a nonsettling, almost opaque, gelatinous precipitate indicates Irish moss. Carry out confirmatory test for Irish moss.

A small amount of flocculent precipitate or cloudiness in the cold and a definite lemon yellow color on heating identify gum tragacanth.

Color changing during heating to yellow, then to green, and finally to gray indicates agar. Carry out confirmatory test for agar.

If the mixture becomes cloudy or forms a gel on heating, but becomes clear on cooling, methylcellulose is indicated. Carry out confirmatory test for methylcellulose.

An opaque flocculent precipitate which may tend to redisperse on heating and reprecipitate on cooling indicates starch. Carry out confirmatory test for starch.

Precipitates which disappear when the barium hydroxide is thoroughly mixed with the sample may be disregarded at this point.

II. If the material has not been identified, mix a fresh 3- to 5-ml. aliquot of sample with 1 volume of saturated barium hydroxide. Observe whether there is an immediate precipitation and examine again after standing 5 minutes at room temperature.

Table IV. Precipitation Reactions Used as Basis of Proposed Procedure for Identification of Gums

(Reactions used as identification tests within heavy lines)

Gum	1/5 Vol. 2.5% $CaCl_2$	1/5 Vol. 2.5% $CaCl_2$ + 1/5 Vol. 3 *N* NH_4OH	1 Vol. 3 *N* NaOH	1/10 Vol. Saturated $Ba(OH)_2$: Cold	1/10 Vol. Saturated $Ba(OH)_2$: Heated	1 Vol. Saturated $Ba(OH)_2$	1/5 Vol. Basic Lead Acetate	Basic Lead Acetate + 1/5 Vol. 3*N* NH_4OH	Confirmatory Tests
De-esterified pectin	Gelatinous ppt. or gel		Gelatinous or flocculent ppt., yellow color on heating	Gel or gelatinous ppt.	Ppt. and soln. turn yellow	As for 1/10 vol.	Gels	As for lead acetate alone	Gelatinous ppt. when acidified with mineral acids
Alginate	Gelatinous ppt. or gel		Clear yellow soln. on heating	Gel or gelatinous ppt.	Ppt. and soln. turn yellow	As for 1/10 vol.	Gels	As for lead acetate alone	Gelatinous ppt. when acidified with mineral acids
Pectin		Gelatinous ppt. forms slowly	Gelatinous or flocculent ppt., yellow color on heating	Gel or gelatinous ppt.	Ppt. and soln. turn yellow	As for 1/10 vol.	Translucent gel	As for lead acetate alone	
Irish moss	Some samples may give flocculent ppt.			Gelatinous ppt. or gel, almost opaque	Ppt. may flocculate with prolonged heating	Gelatinous ppt. or gel	Gelatinous ppt. or gel	As for lead acetate alone	Forms blue fibrous ppt. with aqueous methylene blue
Tragacanth				May be small amount of ppt.	Lemon yellow color	Flocculent ppt.	Flocculent ppt.	As for lead acetate alone	
Agar				Nil	Becomes yellow then green and gray	Nil	Flocculent ppt.	Gels	Gives blue or black stain with tincture of iodine
Methylcellulose				Nil	Becomes turbid or forms gel; becomes clear on cooling	Nil	Nil	Gels	Aqueous dispersions are not precipitated by alcohol, but form gel or become cloudy when heated
Starch				Opaque flocculent ppt.	May redisperse	Flocculent ppt.	Flocculent ppt.	Very heavy flocculent or gelatinous ppt.	Blue stain with I_2-KI reagent
Carboxymethylcellulose				Ppt. dissolves on shaking	Nil	Flocculent ppt.	Gels	As for lead acetate alone	Forms clotted ppt. with $CuSO_4$
Locust*						Clotted opaque ppt.	Opaque gel	As for lead acetate alone	Gels with 1/5 vol. 4% borax
Karaya						Flocculent ppt., forms slowly	Flocculent ppt.	As for lead acetate alone	Swells and stains pink with ruthenium red test solution
Arabic (acacia)							Voluminous opaque ppt.	As for lead acetate alone	Readily soluble in water at room temperature
Ghatti							May be small amount of flocculent ppt.	Voluminous opaque ppt.	Fine ppt. with 4 vol. of alcohol. Arabic also gives fine ppt.
Gelatin									Gives fine yellow ppt. when added to saturated picric acid

* Locust bean gum and guar gum give identical reactions and cannot be distinguished on basis of these tests.

Table V. Samples Examined by Proposed Identification Procedure

Gum	Type	No. of Samples
Sodium pectate	Powdered	1
Pectic acid	Granular	1
Pectin	Powdered	2
Sodium alginate	Powdered	5
Irish moss	Powdered	2
Tragacanth	Powdered	1
Agar	Shredded	1
	Granular	3
Methylcellulose	Fibrous	2
Starch	Whole wheat flour	1
	Tapioca flour	1
	Soluble starch	3
	Cornstarch	1
	Amioca (amylopectin)[a]	1
	Clear jel[a]	1
	Clear-Flo-II (sodium salt of starch acid ester)[a]	1
	Dry-Flo (starch ester)[a]	1
	Vulca (starch ether)[a]	1
	Melojel[a]	1
	Nu-film (starch acid ester)[a]	1
Sodium carboxymethylcellulose	Powdered	1
Locust bean (carob)	Powdered	7
Guar	Powdered	1
Karaya	Powdered	1
Arabic (acacia)	Powdered	2
	Lump	1
Ghatti	Powdered	1
	Lump	1
Gelatin	Granular	2

[a] Products of National Starch Products, New York, N. Y.

A voluminous opaque, stringy precipitate which tends to form a clot indicates locust bean gum. This precipitate may appear flocculent if the mixture is shaken vigorously. Carry out confirmatory test for locust bean gum.

A voluminous opaque flocculent precipitate which forms immediately indicates carboxymethylcellulose. Carry out confirmatory test for carboxymethylcellulose.

An opaque flocculent precipitate which forms slowly and is not voluminous indicates gum karaya. Carry out confirmatory test for karaya.

Group C. If the sample has not been identified it may be gum arabic, gum ghatti, or gelatin.

I. Mix a fresh 3- to 5-ml. aliquot of sample with 1 ml. of basic lead acetate solution. Immediate formation of a voluminous opaque precipitate indicates gum arabic.

If there was only a small amount of flocculent precipitate, or no precipitate, with the basic lead acetate add 1 ml. of 3.0 N ammonium hydroxide to the lead-containing sample. A voluminous opaque flocculent precipitate indicates gum ghatti. If there is no precipitate, the sample probably is gelatin. Carry out confirmatory test for gelatin.

CONFIRMATORY TESTS

ALGINATES AND DE-ESTERIFIED PECTINS. Add 0.2 volume of 3 N hydrochloric acid (or other mineral acid) to 3 to 5 ml. of the sample. A gelatinous precipitate confirms alginates or de-esterified pectin.

IRISH MOSS. Add 2 to 3 drops of 0.5% methylene blue in water to 1 ml. of the sample solution. Precipitation of purple-stained fibers confirms Irish moss.

METHYLCELLULOSE. Mix 5 ml. of sample with 25 ml. of 95% alcohol and 2 to 3 drops of saturated sodium chloride. No precipitate confirms methylcellulose.

AGAR. Precipitate gum from 5 ml. of sample with alcohol and stain with tincture of iodine (*3*). Starch is also stained blue by this reagent.

STARCH. Add 1 to 2 drops of the iodine potassium iodide test solution to 1 ml. of sample. A blue or purple color confirms starch. Some samples of gum tragacanth may give a faint blue test here.

CARBOXYMETHYLCELLULOSE. Add 2 ml. of 1.0 M cupric sulfate to 3 to 5 ml. of sample solution. An opaque, slightly bluish, clotted precipitate confirms carboxymethylcellulose.

LOCUST BEAN GUM. Add 1 ml. of 4% borax to 3 to 5 ml. of gum solution. If mixture gelatinizes, locust bean gum is confirmed. Guar gum also forms a gel here.

KARAYA. Precipitate gum from 5 ml. of solution with alcohol and stain with ruthenium red (*3*). If sample swells considerably and is stained pink, karaya is confirmed.

GELATIN. Add 2 to 3 drops of gum solution to 2 ml. of saturated picric acid. A fine yellow precipitate confirms gelatin.

DISCUSSION

The proposed procedure has been tested with the gums listed in Table V. It was possible to identify the modified starches as starch products, because the solubility of all these materials is decreased by barium hydroxide and all give positive tests with the iodine–potassium iodide reagent. It is possible that not all the thickening agents or gums employed in food products at the present time have been included in this study. Cherry gum and quince seed gum, for example, have been suggested in the literature for use in foods. However, the proposed procedure includes all the gums that were available during the investigation.

The proposed scheme for identification of stabilizing and thickening agents is applicable only when they have not been mixed with other materials. To identify thickening agents in foods by this method it would first be necessary to separate them from the foods, but separation techniques might alter the reaction characteristics of the gums. Much work has been done on methods for the separation and detection of gums in particular foods, such as mayonnaise and French dressing (*2, 7, 10, 11*), soft curd cheese (*1, 8, 10, 11, 13, 19*), tomato products (*10, 13*), starchy foods (*21*), cacao products (*13, 17, 23*), ice cream and frozen desserts (*9–11*), canned chicken (*10*), and meat products (*12*). Additional references to methods for separating gums from foods may be found in reviews by Jacobs and Jaffe (*15*) and Mantell (*16*). The emphasis in most procedures has been on detection of the gums without identification. However, Wyler (*24*) has outlined methods for the detection and identification of locust (carob) bean gum, methylcellulose, carboxymethylcellulose, starch, pectin, and alginate in meat products. Thus identification tests proposed in the present paper may be useful for the identification of gums separated from foods by methods already described in the literature. However, it is probable that many special techniques will be required for the analyses of particular combinations of foods and thickeners. A great deal more work must be done before it will be possible to identify all of the gums in the various foods in which they may be used.

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THE BEHAVIOR OF CAROB GUM IN THE GASTROINTESTINAL TRACT OF MAN

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CONSTIPATION IS PROBABLY the most common symptom suffered by mankind. To be impressed by the implication of this statement one need merely consider the multitude of means man has employed to obtain relief from constipation: enema, colonic irrigation and suppository; salines, aloes, cascara, senna, licorice and rhubarb; calomel, phenolphthalein and sulfur; oils (mineral and castor), gums and so forth. The tragedy of constipation is that due to the ready availability of all these means, the sufferer for a time finds no difficulty in treating himself, little realizing that his symptom may be the first indication of some organic disease like bowel malignancy. The physician should prescribe for every patient with constipation. It is the purpose of this communication to present a new preparation which promises to be highly useful in this respect.

The preparation under discussion is called "Vacuosa."* Its active principle, the hemicellulose of the carob seed, is literally as old as the hills, being derived from the bean (fruit) of the Locust Tree, known in biblical times as St. John's Bread Tree. This leguminous plant is native to the shores of the Mediterranean. The gum from the carob bean has marked hydrophilic potency and thus fits into the same category as the vegetable gums of agar, acacia, tragacanth, karaya and psyllium seed, all of which are transformed to a colloidal state. There are, however, conspicuous differences in the rate of such transformation, the consistency (viscosity) of the colloid produced and the volume of the gel mass.

Comparative studies of the physical properties of the gums in common use have been published by Badosa, Serrallach and Monroset (1). They found by in vitro experiments that carob gum placed in water swelled into a colloid mass much more efficiently when shaken for an hour than when left standing. The degree of swelling was not appreciably influenced by changes in pH nor by gastric or duodenal juices. The viscosity of the gel formed by carob gum was about 9 times greater than that produced by other gums. Finally, the hemicellulose of the locust bean was apparently not subject to the digestive process, since incubation with digestive juice at 37°C. showed that no reducible sugars could be detected.

These studies have been extended in the laboratory of the author. It was observed that 10 cc. of carob gum granules, stirred every 5 minutes in a graduate containing 500 cc. of water at 37°C. (thus crudely imitating peristaltic agitation in the gastrointestinal tract), in 4 hours will progressively swell to reach a maximum bulk of 77 cc. Approximately the same rate of increase in volume occurs in a 0.2 N NaOH solution; however, in a 0.2 N HCl solution the final volume of gel after 4 hours is 55 cc. At the end of 1 hour there is no apparent difference in volume due to the pH of the media.

METHODS AND RESULTS

The principal purpose of this investigation was to determine the behavior of carob gum ("Vacuosa") in the gastrointestinal tracts of human beings.

Initially, attempts were made by using roentgenological techniques to identify any space-occupying mass within the colon after ingestion of "Vacuosa" Pelloids, employing barium suspensions as a contrast medium. The barium suspension, consisting of 2½ heaping teaspoonfuls of the powder in 8 oz. of water, was given at 10 P.M., and a flat plate of the abdomen was taken at 10 A.M. the following morning. This film served as a control. The procedure was repeated 2 days later in the same fashion except that, in addition, the subject took 2 heaping teaspoonfuls of "Vacuosa" with water immediately following the barium. This type of experiment was performed 12 times on 8 individuals with slight variations in technique. The differences produced by "Vacuosa" in the size of the lumen and character of the haustra of the large bowel were not conclusive with this technique.

The second phase of x-ray studies was devised so as to follow "Vacuosa" in its passage through the entire gastrointestinal tract. In view of the results in the

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initial experiences it was considered desirable to have the radiopaque material more intimately incorporated with the carob gum. Therefore, Pelloids containing equal parts of "Vacuosa" and barium were prepared. Two level teaspoonfuls of this experimental preparation weighed 8 grams as opposed to 6.7 grams for plain "Vacuosa."

As a pilot test, 2 heaping teaspoonfuls of the "Vacuosa"-barium Pelloids were given to 9 fasting volunteers at 8 A.M. Abdominal x-ray films were taken at ½ hour intervals for 5 successive times on two of the subjects, and at hourly intervals for 3 hours on the other seven subjects. It was found that disintegration of the Pelloids was uniformly complete after the third hour, as judged by the change from small, discrete shadows to large, diffuse ones.

Another series of 10 cases was studied using the method outlined in the pilot test, except that films were taken at 1, 3, 8 and 24 hour intervals. None of the individuals had the laxative habit. Six were men, four women. They all had white collar jobs. Two typical examples of the results are shown in the left columns of Figures 1 and 2. In both these cases the pellet aspect of the "Vacuosa"-barium is still apparent in the three hour films. The 24 hour films reveal the thoroughness with which the carob gum preparation mixes with the feces in the large bowel.

In order to gain perspective for judging the in-vivo behavior of "Vacuosa," parallel x-ray studies were made using mixtures of psyllium seed gum with barium and karaya gum with barium, prepared exactly like the "Vacuosa"-barium mixture. The same 10 subjects were chosen so that results would be comparable. Figures 1 and 2 present all the films for 2 of these cases. The large mass of data obtained from the entire series is depicted graphically in Figure 3. The films for each case were plotted in the manner shown, and then the averages were taken for the composite picture. It will be seen that the carob gum preparation was the slowest to disintegrate and the slowest to pass through the gastrointestinal tract. The psyllium and karaya gums behaved alike, though the latter showed somewhat faster progress through the intestinal tract.

Laboratory and clinical observations were made on the effects of "Vacuosa" upon the stools of 17 hospitalized patients. Six of this group suffered from chronic constipation and two, from recurrent diarrhea. The remaining nine patients were considered to have normal bowel habits. The oral dose was 2 heaping teaspoonfuls of the Pelloids with 2 glasses of water taken at bedtime. Five consecutive stool examinations were made as regularly as possible, both before and during the administration of "Vacuosa." Each specimen was weighed and measured volumetrically. The gross physical properties were carefully noted. The stools passed during the period of medication were submitted to microscopic examination as well.

Since this series is small, only the total figures are indicated.

TABLE I

	CONTROL	"VACUOSA"
Number of stools	56	47
Total weight of stools	9,167 Gm.	8,457 Gm.
Total volume of stools	9,935 cc.	10,154 cc.
Average weight	174.87 Gm.	193.34 Gm.
Average volume	185.40 cc.	225.34 cc.

In seven stools "Vacuosa" Pelloids were definitely identified grossly; and in fifteen, microscopically. There was an average gain of about 20 grams per stool, or 10.2 per cent, as shown in Table I. The stool bulk increase averaged about 40 cc. per unit, or 22.0 per cent, after "Vacuosa." The stool containing carob gum tended to be soft, gelatinous and homogeneous, while its control counterpart was either hard and formed or loose and mushy. Liquid stools were not altered by this preparation. The typical "Vacuosa" stool had such consistency that it did not settle out when placed in a container but maintained for hours the shape in which it was passed. The stool mass was characterized by a mucilaginous coating on its surface which often revealed tiny gelatinous particles resembling fine tapioca pudding. These particles under the microscope were seen to be remnants of the "Vacuosa" Pelloids.

In addition to the 44 patients mentioned above, "Vacuosa" was given to 12 private ambulatory patients with chronic constipation. Some patients of this latter group have taken "Vacuosa" regularly for 2 years. It has been interesting to note that the majority of the normals and the constipated alike, volunteer the comment that their stools become "easier to pass" and none experienced epigastric distress or abdominal colic after taking "Vacuosa." The full benefits of "Vacuosa" may not be obtained, however, until treatment has been in progress for several days.

DISCUSSION

Both the x-ray studies and the stool examinations have indicated that the colloidal gel resulting from the disintegration of the "Vacuosa" Pelloids permeates the fecal mass in the colon and mixes thoroughly with it. The greatest effect on the feces is the alteration in consistency. There is little actual increase in stool weight.

The comparative x-ray studies of carob, psyllium and karaya gums illustrate the important fact that carob gum does not disintegrate into a gelatinous state until it reaches the large bowel whereas the other two gums are transformed much more quickly and at a higher level of the gastrointestinal tract. Subsequent clinical observations indicate that there is no interference with normal digestion and less sense of bloating or distention with the use of carob gum preparations than the others. This may well be due to the fact that carob gum Pelloids maintain their integrity through the small bowel and do not increase the rate of peristalsis and the rate of passage, alterations which frequently result from the rapid swelling seen with other gums.

No instance of any allergic reaction attributable to "Vacuosa" has been experienced. Sensitivity to other vegetable gums has been reported by Gelfand (2). "Vacuosa" Pelloids have ready patient acceptability and are easy to swallow.

SUMMARY

1. The physical properties of carob seed gum are discussed.

2. A study of the behavior of this gum in the gastrointestinal tracts of human beings is described.

a) Twelve attempts were made by x-ray to demonstrate increased colonic contents 12 hours after volunteers took 2 heaping teaspoonfuls of "Vacuosa" orally.

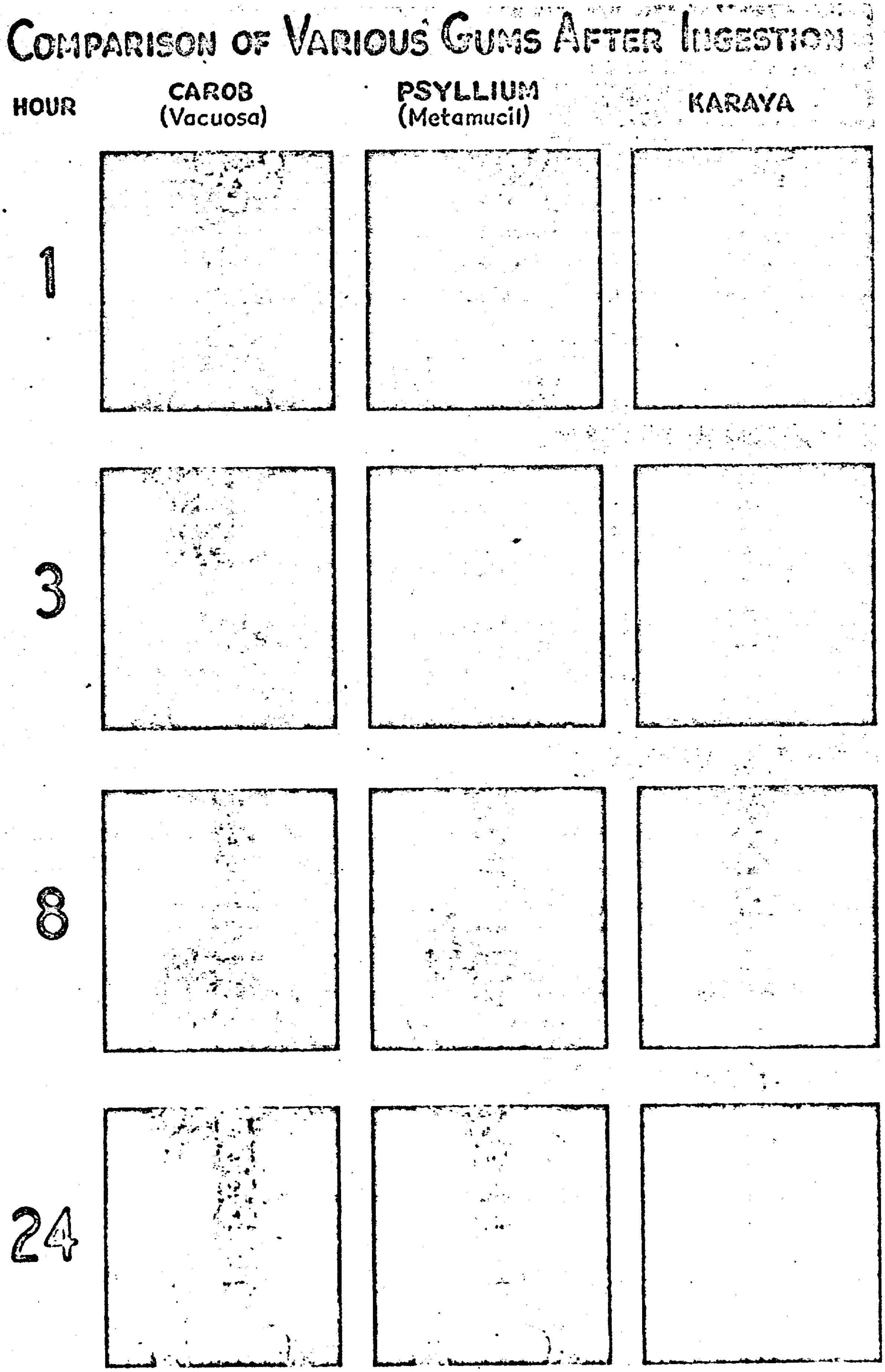

Fig. 1 Patient F.M. Note difference in rate of passage and disintegration.

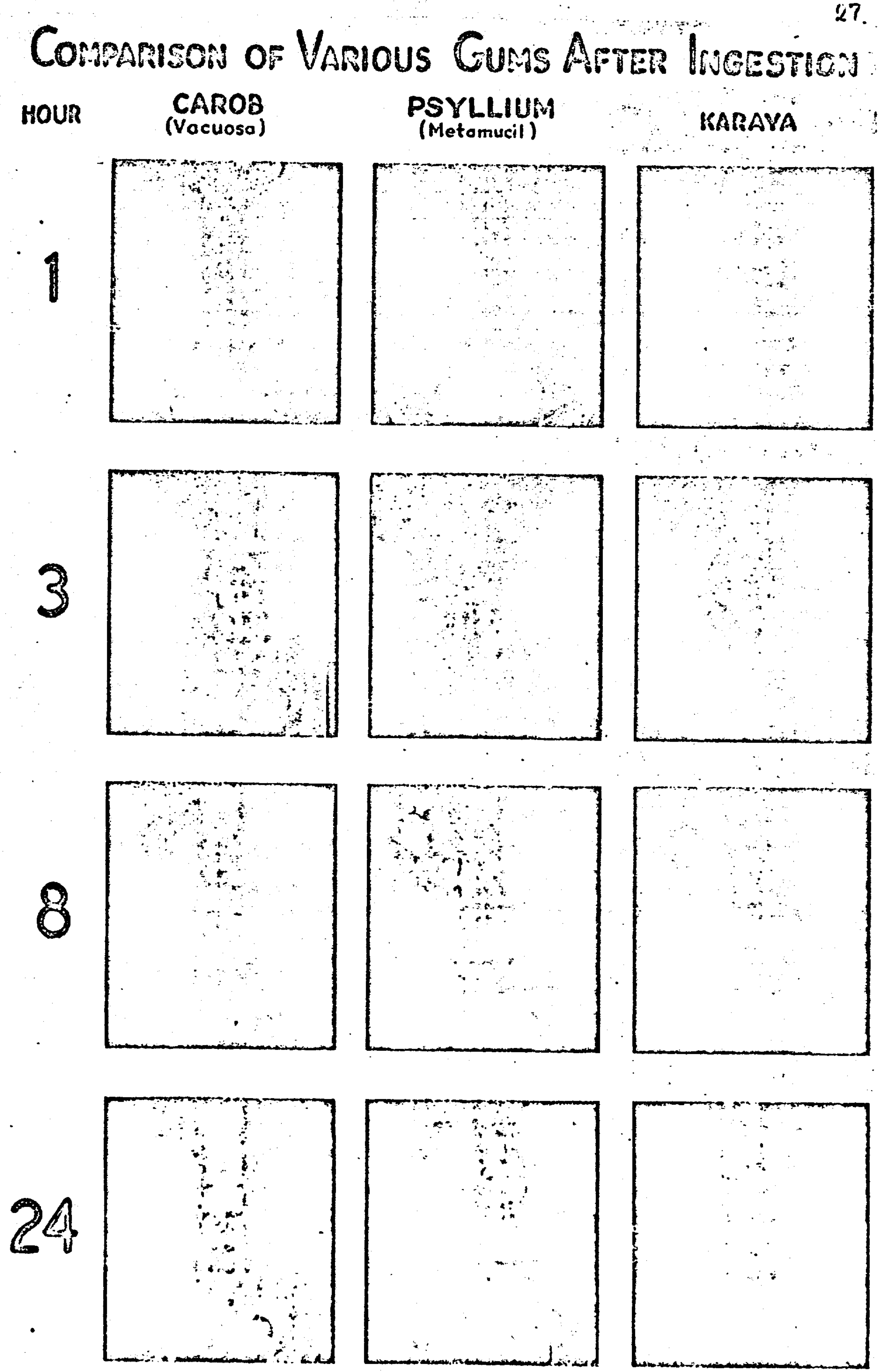

Fig.2 Patient R.L. Note difference in rate of passage and disintegration.

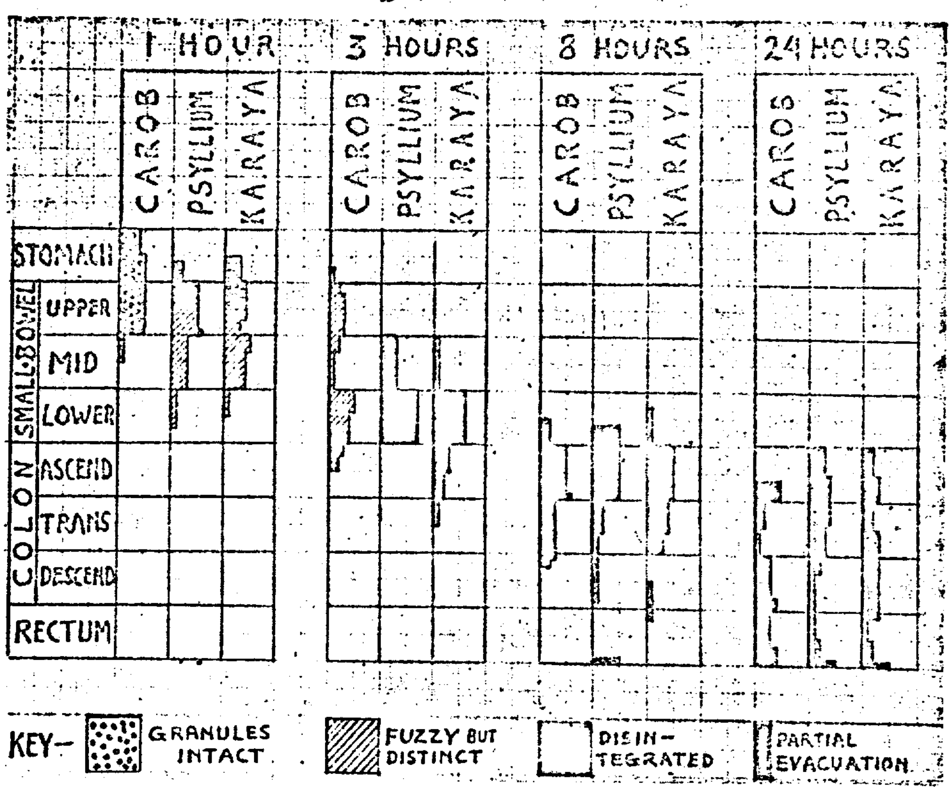

Fig. 3. A graphic representation of the results of x-ray studies made on 10 individuals who took 3 special gum preparations by mouth.

b) The progress of "Vacuosa"-barium Pelloids ingested by 19 subjects was followed from stomach to rectum by x-rays with special reference to position and time of disintegration.

c) Ten members of this group were similarly studied twice again, using psyllium gum-barium and karaya gum-barium mixtures. The x-rays of 2 cases and a chart of the average results are presented.

d) The quantitative and qualitative effects of "Vacuosa" upon the stools of 17 hospitalized patients are reported.

3. A brief discussion is given concerning the advantageous features of "Vacuosa," which encourage its further clinical use.

CONCLUSIONS

1. The swelling capacity of carob gum in a neutral or alkaline solution to more than 7 times its original volume has been demonstrable in vitro.

2. "Vacuosa"-barium Pelloids observed serially by x-ray after ingestion by normal individuals were seen to maintain their identity for the first 3 hours in the stomach and small bowel. The disintegration time for "Vacuosa" Pelloids approximated the ideal physiological timing in that they did not reach their maximal colloidal mass until they were in the colon, whereas psyllium gum-barium and karaya gum-barium Pelloids disintegrated much sooner and passed more rapidly through the upper gastrointestinal tract. In the colon and rectum, however, all gums appeared to behave similarly.

3. "Vacuosa" is an acceptable, bland, non-allergenic substance with the capacity of forming a colloidal gel which clinically affects stools in a way which makes them "easier to pass" and assists in promoting normal stool habits by causing an increase in bulk which is soft, homogeneous and non-irritating.

Footnote. X-rays produced in Figures 1 and 2 were taken at Columbia Hospital, Milwaukee.

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The Effect of Polysaccharides on Energy Utilization, Nitrogen Retention and Fat Absorption in Chickens

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A DEPRESSION in the growth of rats was observed when 5% of agar agar or Irish moss was added to the diet of rats (Nilson and Schaller, 1941), while Vaughan *et al.* (1962) reported that carrageenin inhibited the *in vitro* digestion of proteins by pepsin. Booth *et al.* (1963) found that the digestion in rats of agar agar was 21%, pectin 19% and guar gum 76% when fed at levels of 15%, 10% and 6%, respectively.

Ershoff and Wells (1962) reported no significant growth depression in rats with 10% pectin, guar gum, locust bean gum or carrageenan. These materials, however, reduced plasma and liver cholesterol when 1% of cholesterol was fed in the diet. Fisher *et al.* (1964) reported that pectin reduced blood cholesterol in chicks fed cholesterol and Fisher *et al.* (1965) reported further that pectin reduced blood cholesterol in chicks only when cholesterol was fed.

Vohra and Kratzer (1964), and Anderson and Warnick (1964) reported that several natural polysaccharides depressed the growth of chicks when fed in the diet. The growth depressing effects of some of these polysaccharides were destroyed when these materials were treated with suitable enzymes. Riccardi and Fahrenbach (1965) conducted experiments with chicks fed diets containing 3% cholesterol. Under their conditions 3% of guar gum or carrageenan caused a 50% reduction in plasma cholesterol, but there was no gross toxicity as measured by survival, body weight depression or reduced food consumption.

The purpose of the present study is to investigate the mechanism of action of certain deleterious polysaccharides when fed to chickens by specifically studying their effect on energy utilization, nitrogen retention and fat absorption.

EXPERIMENTAL

The basal diets (Table 1) were based on commonly used natural feedstuffs but also contained 0.3% chromic oxide as an indicator to relate feed to feces quantitatively. Additions of polysaccharides were made at a level of 2% at the expense of milo in the conventional basal diet. The protein content of the high fat diet was increased in proportion to the increased level of fat on a calculated metabolizable energy basis. The total weight of the diet was not adjusted to 1 kg. The high protein (approximately 30%) diet was not adjusted in this way

TABLE 1.—*Composition of the experimental diets*

Ingredient	Low fat basal	High fat basal[1]	High protein basal
	gm./kg.	gm.	gm./kg.
Ground milo	270	—	—
Ground yellow corn	300	—	335
Corn starch	—	173	—
Soybean meal (50% protein)	200	288	415
Fish meal (65% protein)	75	105	75
Dried whey	50	50	50
Dehydrated, alfalfa meal (20% protein)	40	40	40
Soybean oil	—	100	20
$CaHPO_4 \cdot 2H_2O$	25	25	25
Cr_2O_3 mix[2]	10	10	10
Sodium chloride[3]	5	5	5
Vitamin mixture[4]	5	5	5
Test material	20	20	20

[1] The total mix was not adjusted to 1 kg.
[2] Mixed in wheat flour dough, baked and ground powder supplied 3 gm. Cr_2O_3.
[3] Iodized sodium chloride contained 0.025 gm. $MnSO_4 \cdot H_2O$/5 gm.
[4] Supplies: riboflavin, 1.1 mg.; niacin, 1.1 mg.; Ca-pantothenate, 1.1 mg.; choline chloride, 5.5 mg.; folic acid, 33.3 µg.; vitamin A, 7,500 I.U.; vitamin D_3, 1,270 I.C.U. (exp. 1 and 3) or 127 I.C.U. (exp. 2); vitamin E, 68 I.U. and bran to make 5 grams.

TABLE 2.—*Effect of 2% levels of various polysaccharides on weight gain, feed consumption, nitrogen retention, and fat absorption in chicks, and the metabolizable energy of the feed containing 1,270 I.C.U./kg. vitamin D_3 (Experiment 1)*

Supplement	Weight gain gm.	Feed intake gm.	Feed / gain	% N retention	% Fat absorbed	Metabolizable energy kcal./gm.
Cellulose	299^{d5}	513	1.72^{a5}	51^{bcde5}	76^{cd5}	2.93^{bc5}
Guar gum	149^{a}	358	2.42^{d}	33^{a}	64^{a}	2.44^{a}
Gum carob	180^{ab}	355	1.98^{b}	53^{def}	82^{d}	2.90^{bc}
Gum karaya	229^{bc}	447	1.95^{b}	45^{b}	74^{bcd}	3.08^{c}
Pectin	231^{bc}	410	1.78^{a}	47^{bcd}	79^{cd}	2.89^{bc}
Me-Et-Cel[1]	304^{d}	543	1.79^{a}	58^{f}	79^{cd}	3.13^{c}
Me-Cel[2]	306^{d}	543	1.76^{a}	56^{ef}	76^{cd}	2.92^{bc}
HO-Pr-Cel[3]	275^{cd}	514	1.78^{a}	53^{cdef}	76^{abc}	2.89^{bc}
CM Cel (4 HP)[4]	236^{bc}	466	1.98^{b}	46^{bcd}	65^{a}	2.89^{bc}

[1] Methylethycellulose.
[2] Methylcellulose.
[3] Hydroxypropylcellulose.
[4] Carboxymethycellulose.
[5] Statistical significance at 1% level is denoted by different letters in a column.

and had a higher protein to energy ratio than the other two diets.

The vitamin D_3 supplements were added to the diets to provide 1270 I.C.U./kg. which is six fold more than the requirement of chicks for this vitamin (200 I.C.U./kg. diet; N.R.C., 1966). However, due to an error in mixing, the level of vitamin D_3 added to the diet in experiment 2 was 127 I.C.U./kg. diet, thus giving a diet marginally deficient in this vitamin.

Day-old Arbor Acres chicks were divided into duplicate groups of 10 chicks each of equivalent weight distribution. The birds were housed in wire cage batteries. Feed and water was supplied *ad libitum* and the experiments were continued for 3 weeks. The method of Dansky and Hill (1952) was used for determining apparent nitrogen retention and fat absorption while metabolizable energy was measured by the method of Hill and Anderson (1958). Data were analyzed statistically by the multiple range method of Duncan (1955).

The polysaccharides tested in this report were either naturally occurring gums or derivatives of cellulose commonly used in food industry. These included guar gum, carob gum, gum karaya, pectin, methylethylcellulose, methylcellulose, hydroxypropylcellulose and carboxymethylcellulose—4 HP or 7 HP.

RESULTS AND DISCUSSION

All natural gums depressed growth in the first experiment (Table 2). Feed efficiency was also reduced by all the natural gums with the exception of pectin. Carboxymethylcellulose in this experiment produced a significant growth depression and reduction in feed efficiency but methylethylcellulose, methylcellulose or hydroxypropylcellulose had no growth depressing properties for chicks.

Nitrogen retention (Table 2) was significantly reduced by guar gum while gum carob, gum karaya and pectin caused no significant change from the control containing cellulose. Fat absorption was also reduced by guar gum but not the other naturally occurring polysaccharides. Metabolizable energy, likewise, showed a significant reduction with guar gum. Carboxymethylcellulose (4 HP) produced a slightly lower nitrogen retention but the differences was not statistically significant. Carboxymethylcellulose caused a significant reduction in fat absorption but

TABLE 3.—*Effect of fat and protein level on response of chicks to polysaccharides in diets containing a marginal level of vitamin D_3 (127 I.C.U./kg. diet). (Experiment 2)*

Type of diet	Supplement	Body weight gain, gm.	% Apparent N retention	% Fat absorption	% Bone ash	Pancreas weight, mg./100 gm. body weight
Low fat	Cellulose	311^{e2}	46.9^{de1}	63.4^{bc1}	44.4^{c1}	340^{a2}
	Gum karaya	289^{cde}	44.4^{de}	60.3^{bc}	41.0^{c}	355^{a}
	CM Cel (7 HP)	247^{bcd}	49.2^{e}	53.0^{a}	32.6^{ab}	370^{ab}
High fat	Corn	297^{cde}	51.2^{e}	87.9^{ef}	41.1^{c}	345^{a}
	Cellulose	297^{cde}	44.9^{de}	92.6^{f}	41.3^{c}	375^{ab}
	Pectin	246^{bc}	44.1^{cd}	82.8^{e}	35.2^{ab}	375^{ab}
	Guar gum	178^{a}	36.1^{bc}	63.5^{bc}	31.2^{a}	390^{ab}
	CM Cel (7 HP)	219^{ad}	41.0^{cd}	86.1^{e}	—[3]	445^{bc}
High protein	Cellulose	300^{de}	49.3^{e}	85.1^{e}	41.6^{c}	375^{ab}
	Guar gum	194^{ab}	23.7^{a}	65.0^{c}	32.6^{ab}	490^{c}
	CM Cel (7 HP)	236^{b}	30.4^{ab}	72.7^{d}	—[3]	425^{abc}

[1] Statistical significance at 1% level by Duncan's method.
[2] Statistical significance at 5% level by Duncan's method.
[3] Not determined.

there was no effect on metabolizable energy of the diet by the addition of any of the cellulose derivatives.

The deleterious effect of guar gum upon growth, nitrogen retention, fat absorption and metabolizable energy raised the question whether these responses would be mitigated by either raising the energy content of the diet with additional soybean oil while maintaining the energy/protein ratio the same as in low fat diet, or increasing the level of protein alone.

The results of the experiment in which guar gum was fed in high fat or high protein diets are given in Table 3. The diets were marginally deficient in vitamin D_3. The chickens fed 2% carboxymethylcellulose (7 HP), pectin, and guar gum in all diets containing only 127 I.C.U./kg. vitamin D_3 developed rickets which was not noticed with other polysaccharides. The dietary requirements for vitamin D_3 are believed to be 200 I.C.U./kg. The rickets were confirmed by reduction in bone ash for some treatments (Table 3), and appeared to be associated with the growth depressing polysaccharides only. A marked reduction in growth of chickens was observed for carboxymethylcellulose, guar gum, and pectin on low fat, high fat or high protein diets. The depression in growth caused by gum karaya was statistically non-significant. Nitrogen retention and fat absorption data were approximately similar to those of the previous experiment with the exception that guar gum interfered with these parameters more on the high fat diet than was noted previously. Pancreatic weight was significantly increased when the diets contained guar gum in high protein diets or carboxymethylcellulose (7 HP) in high fat diets. In other treatments, there were no significant differences in the pancreatic weights as expressed in terms of the body weight of chickens.

The vitamin D_3 level of the diets used in experiment 3 was again increased to 1270 I.C.U./kg. to determine whether the growth depressing polysaccharides caused rickets only in diets marginally deficient in vitamin D_3. No rickets were observed with any of the treatments in this experiment which is again confirmed by no statistically significant differences in any of the bone (tibia) ash samples (Table 4). As in the

TABLE 4.—*Effect of fat and protein level on response of chicks to polysaccharides containing high levels of vitamin D_3 (1,270 I.C.U./kg. diet) (Experiment 3)*

Type of diet	Supplement	Body wt. gain gm.	% N retained	% Fat absorbed	Pancreas wt. mg./100 g. body wt.	% Bone ash
Low fat	Cellulose	336^{ef}[1]	48^{cd}[1]	70^{ab}[1]	400^{ab}[1]	43.6^{a}[1]
	Gum karaya	248^{bc}	49^{cd}	74^{b}	495^{abc}	42.6^{a}
	CM Cel (7 HP)	243^{abc}	39^{b}	64^{a}	550^{cd}	42.8^{a}
High fat	Corn	355^{f}	54^{d}	95^{d}	385^{a}	41.9^{a}
	Cellulose	369^{f}	54^{d}	94^{d}	367^{a}	43.7^{a}
	Pectin	299^{cde}	53^{cd}	89^{cd}	440^{abc}	42.8^{a}
	Guar gum	295^{cde}	52^{cd}	91^{cd}	456^{abc}	41.8^{a}
	CM Cel (7 HP)	211^{ab}	47^{c}	69^{ab}	567^{cd}	43.8^{a}
High protein	Cellulose	322^{ef}	51^{cd}	87^{c}	477^{abc}	43.7^{a}
	Guar gum	188^{a}	30^{a}	75^{b}	639^{d}	41.0^{a}
	CM Cel (7 HP)	273^{cd}	32^{ab}	87^{c}	513^{bcd}	42.9^{a}

[1] Statistical significance at 1% level in a column by Duncan's method is indicated by different letters.

first experiment, gum karaya and carboxymethylcellulose depressed the growth of chickens significantly on low fat diets. The nitrogen retention was depressed significantly only by carboxymethylcellulose but the fat absorption was unchanged. The depression in growth of chickens also took place on high fat diets with pectin, guar gum or carboxymethylcellulose; and on high protein diets with guar gum or carboxymethylcellulose. Nitrogen retention and fat absorption were depressed by carboxymethylcellulose only on high fat diets. Nitrogen retention and fat absorption were reduced by guar gum on high protein diets but only nitrogen retention was affected by carboxymethylcellulose under these conditions. Carboxymethylcellulose caused enlargement of the pancreas on low fat, and high fat diets but not on high protein diets. Guar gum affected pancreas only in high protein diets.

The growth depressing effect of complex polysaccharides which had been reported previously (Vohra and Kratzer, 1964) was confirmed in these studies.

Guar gum, which depressed growth more severely than other natural gums, was shown to depress nitrogen retention, fat absorption and reduce the metabolizable energy of the whole diet. The greatest aggravation of growth was noted when it was fed in a high protein diet, indicating that its greatest effect was upon nitrogen utilization. On the other hand, carboxymethylcellulose was more growth depressing in a high fat diet. It is not possible to make any further generalizations concerning the effect of the polysaccharides on nitrogen retention and fat absorption.

The significant reduction in the metabolizable energy of the diet by guar gum probably represents a combination of the effect on nitrogen retention and fat absorption. It is analogous to the effect observed by feeding gossypol (Hill and Totsuka, 1964). The enlargement of the pancreas observed in groups fed the growth depressing gums is also similar to that noted in birds fed raw soybean meal.

The lowered bone ash observed in groups fed growth depressing polysaccharides in the diet with a marginal level of vitamin D might be explained by a poor absorption of vitamin D_3, creating a deficiency state which could result in poor bone mineralization. Both Fisher *et al.* (1965) and Riccardi and Fahrenbach (1965) have shown interference with cholesterol utilization caused by pectin, carrageenan or guar gum feeding. Fisher *et al.* (1965) showed that blood cholesterol was lowered by pectin only when cholesterol was fed. Leveille and Sauberlich (1966) showed that pectin decreased the absorption of bile acids and cholesterol in the rat. It is likely that effect of polysaccharides on calcification is also due to interference in the absorption of a steroid, vitamin D_3.

SUMMARY

The following polysaccharides were fed to chicks at a level of 2 percent of the diet: cellulose, methylethylcellulose, methylcellulose, hydroxpropyl cellulose, carboxymethylcellulose, guar gum, gum carob, gum karaya and pectin.

The natural gums and carboxymethylcellulose depressed growth. Guar gum caused reduced nitrogen retention, fat absorption and metabolizable energy while carboxymethylcellulose caused reduced fat absorption. Growth depression was not overcome by high-fat or high-protein diets but guar gum aggravated growth more severely on a high-protein diet.

At a marginally low level of vitamin D_3, the tibia ash was reduced by the growth depressing polysaccharides.

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ON THE IDENTIFICATION BY MEANS OF ELECTROPHORESIS AND CELLULOSE ACETATE FOIL DYEING OF GELS & THICKENING SUBSTANCES THAT ARE LEGAL IN SWITZERLAND.

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1. INTRODUCTION

Besides their gelling and thickening properties, gels and thickening substances used in food manufacture or processing also evidence another important characteristic, namely their ability to emulsify and disperse, thereby providing the explanation for their increasingly wide use as stabilizers in the food processing industry.

Article 443 bis of the Federal Food Regulations defines gels and thickening substances as being those capable of yielding aqueous jellies or aqueous, highly viscous solutions already when in low concentrations. (Concerning admissibility of use, see same document, 56, 110, -1956). Even though they are often being used as stabilizers, they are not designated as such in the food regulation. Within the scope of judicial planning concerning food processing in the EEC, namely admissible emulsifiers, stabilizers, gels and thickening substances (WEISS, 1966), stabilizers were defined as such substances suited for maintaining uniform dispersion of two or more immiscible substances. Most gels and thickening substances are included.

Food monitoring efforts have not been wanting to the end of developing practical testing methods for the analysis of gels and thickening substances. Identification is rendered difficult by the fact that these are high-molecular substances for which no easily executed and specific reactions are known, -- except for starch and the alginates. Furthermore they are used in food only in very minute quantities most of the time, because the desired thickening or stabilizing effect often is already achieved for concentrations under 0.5%. The viscometric method (LETZIG, 1934), based on an appreciable viscosity increase of aqueous solutions due to thickening substances, allows an overall indication of any presence of a thickening substance, but does not permit its identification. Various methods are known for qualitative evidence of gels and thickening substances, which among other methods are based on microscopic tests (CZAJA, 1962; BEYTHIEN & DIEMAIR, 1963), on flocculation reactions (LETZIG, 1955) or on paper chromatographic tests of their hydrolysis products (BECKER, 1956; SULSER, 1957; STOLL & PRATT, 1962), that is, of the corresponding monosaccharides. If the gels and thickening substances to be tested are in pure form, they may be easily tested most of the time under the microscope or by the flocculation reaction. If however their presence in food must be shown, in which there is an interfering substance such as egg white material, then the paper chromatographic test of the hyrolysis products is more promising than a flocculation reaction. However there are cases in which a few gels or thickening substances yield similar sugar components after hydrolysis or where such sugars as glucose and galactose (for instance from lactose in milk products) are detected in the paper chromatographic test, so that the chromatogram will not yield an unambiguous answer. Methods for testing the identity of the purity have been disclosed in the literature (ORGANISATION MONDIALE DE LA SANTE, 1964, 1966). Concerning their numerous applications outside food processing, see also GLICKSMAN (1964).

The process of paper electrophoresis has proved itself for gelatin detection in dairy products (see this publication, 56, 110, 1965). However the cellulose acetate foils are preferable to paper as carrier material, since good separation may be achieved in little time and because the foils may be rapidly rinsed in view of little dye absorption (see section 2.4.1). We further made use of electrophoresis for the analysis of gels and thickening substances belonging to the polysaccharide group.

The described testing method further allows -- besides identification of gels and thickening substances -- quantitative evaluation by means of dye comparisons with solutions of known concentrations of the pertinent gels and thickening substances, the comparison dealing with color intensity. Our research has shown this simple and easily executed electrophoresis process followed by dyeing promises to be very useful in the analysis of gels and thickening means.

As regards food products, polysaccharides may be determined after removal of fatty and egg-white components by means of alcohol precipitation, which is followed by polysaccharide concentration and the subsequently described method.

2. ELECTROPHORESIS OF GELS AND THICKENING SUBSTANCES

2.1 The cellulose acetate foil as carrier substance

Filter paper is unsuitable for electrophoresis research in gels and thickening substances. When the gels and thickening substances being tested are made visible after electrophoresis-separation (see section 3.3), the filter paper reacts as a polysaccharide when the particular dyeing method is used, and it reacts positively. We tested glass fiber strips and cellulose acetate foils in lieu of filter paper. The latter proved particularly suitable. When testing gelatine too, the paper electrophoresis method proved very useful, and cellulose acetate foils were preferable to filter paper as carrier material, because the lesser dye adsorption allowed rapid rinsing of the foils (see figures 1a and 1b).

An important advantage of cellulose acetate foil electrophoresis consists in the shorter test duration with respect to paper electrophoresis, or about 15 to 30 minutes for microelectrophoresis (section 2.4.2), so that interferences or secondary effects during separation, such as evaporation of buffer liquid and the resulting undesired concentration increase are only of insignificant magnitude.

2.2 BUFFER SOLUTIONS

The conventional buffer solutions for paper electrophoresis may also be used as conducting electrolytic solutions for cellulose acetate foil electrophoresis. In general, less concentrated solutions are preferred for the latter process. We selected a borate puffer with a pH of 10 for the electrophoresis of the gels and thickening substances of the polysaccharide group, however the sodium-carbonate sodium-hydrogen-carbonate buffer is more suitable when testing gelatine (see section 2.4.3). The buffer solution concentrations are so adjusted that for a terminal potential of 200 volts, a current of less than 1 ma/cm of foil width is generated. Using both buffers demonstrated anodic migration in all gels and thickening substances that were tested, so that they must be coated on the cathode side at the beginning of the test.

2.3 RENDERING GELS AND THICKENING SUBSTANCES VISIBLE.

Following electrophoresis migration, the cellulose acetate foil is removed from the electrophoresis chamber and the position of the gels and thickening substances will be rendered visible by dyeing them. The various gels and thickening substances evidencing different chemical properties, four different dyeing methods are required (see section 3), which may be differentiated through dyeing because the gels and thickening substances migrate at the same rate under electrophoresis.

2.4 RESULTS

2.4.1 COMPARISON BETWEEN CELLULOSE ACETATE FOILS AND PAPER STRIPS

Pherograms I and II in fig. 1a show the results of our electrophoresis test with cellulose acetate foils (4 x 30 cm) in the Elphor-H electrophoresis chamber of GRASSMANN & HANNIG. Except for buffer concentration and the kind of depositing of the substance tested, test conditions are the same as for paper electrophoresis. The amounts of carbonate buffer with pH of 10 and ion size of 0.15 microns are diluted with the same volumes of distilled water. Deposition of substance tested takes place - not with a micropipette - but with a stamp consisting of two parallel platinum laminae absorbing each time 6 microliter of liquid.

It will be noted when comparing figures 1a and 1b that the cellulose acetate foil electrophoresis allows good separation between gelatine and milk eggwhite in much shorter a time than is possible for paper electrophoresis (3 hours in lieu of 14). Furthermore, the foils may be rinsed when dyeing much faster also (20 minutes in lieu of 2 hours.

2.4.2 MICRO-ELECTROPHORESIS OF GELATINE WITH CELLULOSE ACETATE FOILS

The BECKMANN-SPINCO microzone electrophoresis system allows executing simultaneously 8 separations for probe quantities of 0.25 microliter solution of substance , corresponding to 2-10 microgram of the substance tested, on one cellulose acetate foil of 5.5 x 14 cm (see section 4). Deposition of substance tested is performed by means of a microstamp. This allows reducing separation time to 15-30 minutes. Normally the separation time on a cellulose acetate foil 4 x 30 cm in size and with the Elphor-H electrophoresis chamber of GRASSMANN & HANNIG amounts to about 3 hours (see section 2.4.1)

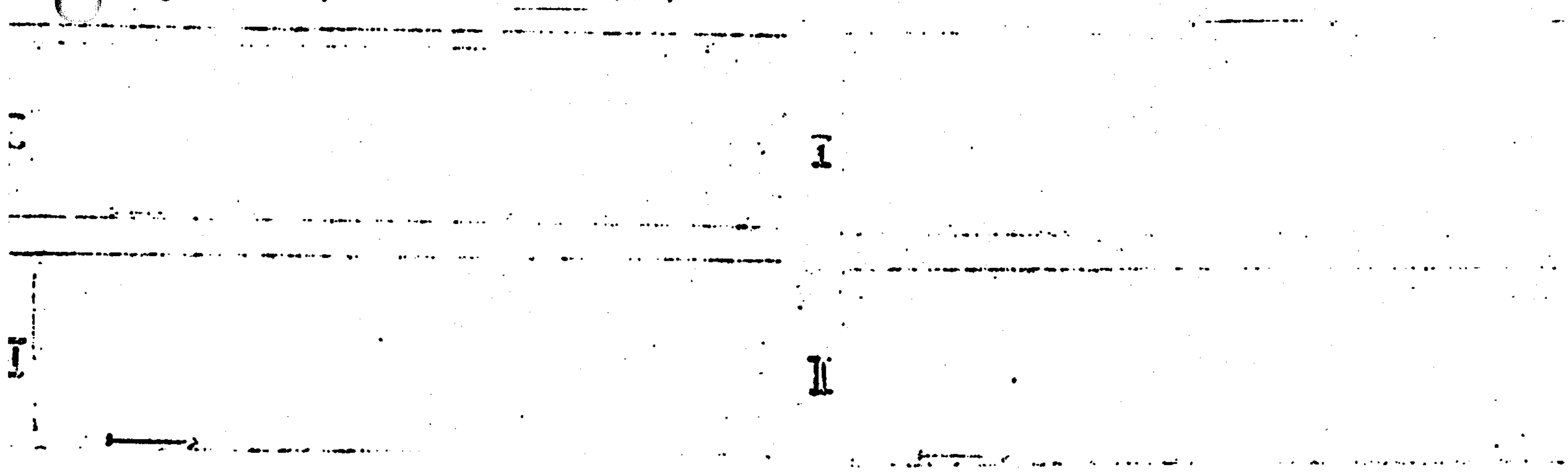


fig. 1a: electrophoresis on cellulose acetate foils

fig. 1b: paper electrophoresis

Fig. 1a: I=gelatin (from 1% gelatin solution, contains about 60 ugm gelatin, deposited with micro-stamp)
II= gelatin and milk egg white (isolated from 0.2% gelatin-content yoghurt, deposited with micro-stamp).
= direction of migration (anodic)
test duration: 3 hours; dyeing plus rinsing: 20 minutes

Fig. 1b: I= gelatin (from 1% gelatin solution, micropipette deposition of 10 ul, contains 100 ugm gelatin)
II= gelatin and milk egg white (isolated from a 0.2%-content-of-gelatin yoghurt, deposited with stamp).
= direction of migration (anodic)
Test duration: 14 hours;
Dyeing and rinsing time: 2 hours

The pherograms show that yoghurt 1 is free of gelatin and that there is gelatin in yoghurt 2. During gelatin isolation, the milk egg white in yoghurt 2 was completely precipitated by means of heat treatment and removed by means of centrifugal action or of filtration. For gelatin concentration, the filtrate will be raised to 10 fold concentration by vacuum evaporation. If the milk egg white is not completely removed, its egg white bands will appear on the pherogram (see picture of self-made yoghurt (b) and that for yoghurt 1). The pherogram shows remaining egg white still capable of migration in yoghurt 1. For the self-made yoghurt sample, -- yoghurt + gelatin --, one may observe completely denatured milk egg white (remaining at the place of deposition) and capable of migrating.

The pherograms therefore show that gelatine tests in dairy products do not require absolute purity in the separated gelatin Even if the extract obtained after yoghurt heat treatment and after gelatin concentration does contain some milk egg white, clean separation of egg whites from gelatin by means of electrophoresis is quite possible.

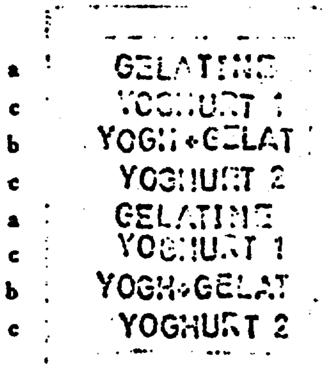


fig. 2

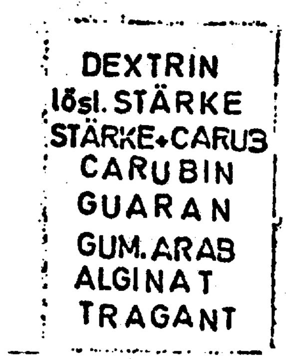


fig. 3
pherogram for PAS positive gels and thickening means.

Fig. 2: shows 8 pherograms from double samples deposited by means of micro-stamp

a) pure gelatin solution as comparison sample; 0.25 ul of 1% solution, corresponding to 2-3 ug of gelatin, deposited
b) extract from self-made yoghurt containing 0.2% gelatin, 10-fold concentration, 0.25 ul deposited, corresonding to 4-6 ug gelatin
c) extracts from commercially obtained yoghurt samples (yoghurt 1 and yoghurt 2); same concentration as in b).

TEST CONDITIONS: 200 volts; carbonate buffer of pH 10 and 0.075 micron ion size; anodic migration; separation time of 25 minutes; amido black dyeing (10 B).

Fig. 3: the amounts deposited are 4-5 ugm for dextrin; 2-3 ugm for soluble starch; 7-8 ugm for carubin; 7-8 ugm for guaran; 7-8 ugm for gum arabic; 2-3 ugm for alginate and 2-3 ugm for tragacanth.
TEST CONDITIONS: 200 volts; borate buffer of Ph 10 and ion size of 0.065 microns; anodic migration; separation time of 15 minutes; PAS dyeing.

2.4.3 MICRO-ELECTROPHORESIS OF GELS AND THICKENING MEANS OF THE POLYSACCHARIDE GROUP

We selected borate buffers with a pH of 10 and ion size of 0.065 microns as the conducting electrolytic solution for the electrophoresis of gels and thickening means of the polysaccharide group. Aside from dyeing, the other test conditions are about the same as those for gelatin (section 2.4.2). Section 4 describes execution of dyeing and of micro-electrophoresis. Fig. 3 shows the pherograms of PAS positive gels and thickening means, fig. 5 shows those made visible following tannin preparation and PAS dyeing, and fig. 6 shows those dyed with toluidine blue O. The pherograms of fig. 4 show the results of electrophoresis separation for mixtures from 2 to 4 different PAS positive gels and thickening means. Section 3 will discuss in detail the dye-affinity of the individual gels and thickening means and their classification according to the dyeing method used.

The pherograms show not only that the gels and thickening means tested migrate at different rates, but also that they are sensitive in different degrees to dyeing.

For the test conditions as selected, the alginate and the tragacanth spots are subject to a remarkable though constant curvature (see also fig. 5 and 6) the cause of which is unknown to us. For micro-electrophoresis with borate buffer, gelatin too evidences this effect (fig. 5), but this is not the case when carbonate buffers are used (fig. 2).

In the pherograms shown in fig. 3, carubin, guaran and gum arabic evidence nearly the same migration rate. If a longer separation time is used, namely 25 minutes, -fig. 4 -, in lieu of 15 minutes, they may be separated from one another.

If the pherograms of figures 5 and 6 are more closely examined, it will be observed that several gels and thickening means migrate nearly at the same rate. Agar-agar, carubin, and tragacanth in fig. 5, carrageen, sodium pectate and alginate in fig. 6 therefore cannot be separated from one another by means of electrophoresis under the given test conditions. In order to identify them, an improved separation may be achieved as in the case of the PAS positive gels and thickening means (fig. 4) by a longer separation time of 20-30 minutes, and they may be further differentiated because of their variable dye-ability (see table 1, section 3).

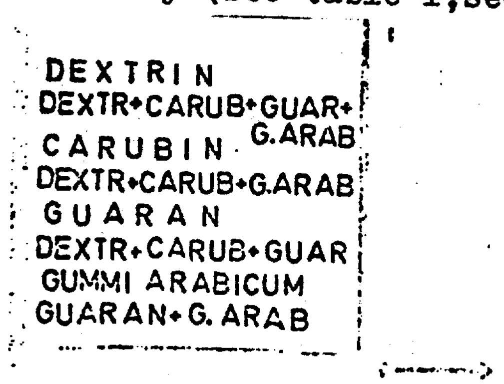


Figure 4

Electrophoresis-separation of mixtures of PAS positive gels and thickening means.

Deposited amount for dextrin was somewhat less (2-3 micrograms in lieu of 4-5), amounts for carubin and guaran were somewhat more (9-10 microgms in lieu of 7-8) than was the case for pherograms in fig. 3. Longer test duration was required for their separation (25 minutes in lieu of 15). Test conditions:

terminal potential: 200 volts
borate buffer: pH = 10; ion size: 0.065 microns
anodic migration
separation time: 25 minutes
PAS dyeing

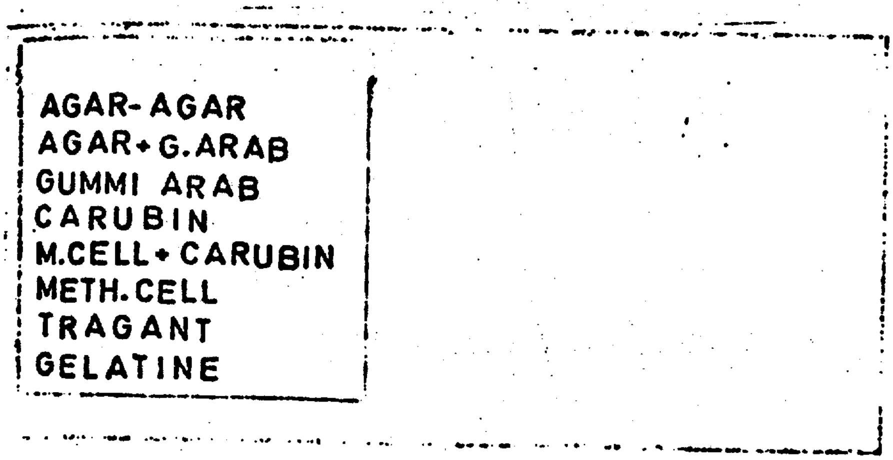


Fig. 5
Pherograms of gels and thickening means rendered visible by means of tannin and PAS dyes preparation.

The deposited amount for agar-agar is 4-5 microgm, for gum arabic 7-8 microgm, for carubin 8-10 microgm, for methyl cellulose 4-5 microgm, for tragacanth 4-5 microgm, and for gelatin 2- microgm.
Test conditions:

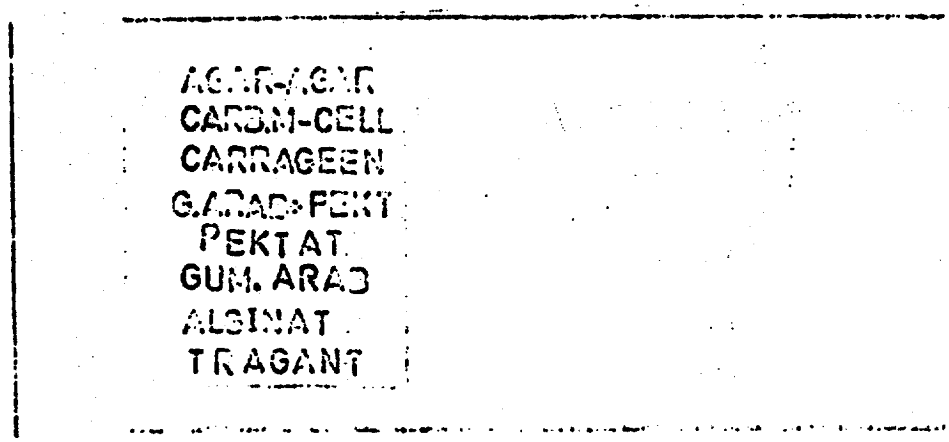
terminal potential: 200 volts
borate buffer pH: 10; ion size: 0.065 microns
anodic migration
separation time: 15 minutes
tannin and PAS dyeing preparation*

SUMMARY

The analysis process described enables identification of individual gels and thickening means as such. In the OFFICIAL METHODS OF ANALYSIS of the AOAC (1965) is listed a method allowing separation of gels and thickening means in food products such as ice cream and mayonnaise after removal of fat and egg white components by means of alcohol precipitation. If this precipitation reaction is positive, then electrophoresis and our dyeing method will allow precise identification of the alcohol precipitate.

Quantitative evaluation may take place by comparing with the color intensities of solutions of known concentrations of the particular gels and thickening means. Coarse estimates may be made by glance alone, photometry being appropriate for precise measurements.

Fig. 6
Pherograms of gels and thickening means with affinity for toluidine blue O



The amounts deposited are, for agar-agar, 4-5 ugm; for carboxy methyl cellulose, 4-5 ugm; for caarageen, 2-3 ugm; for pectate, 4-5 ugm; for gum arabic, 7-8 ugm; for alginate, 2-3 ugm; and for tragacanth, 4-5 ugm.

Test conditions:

terminal potential: 200 volts

borate buffer pH: 10; ion size: 0.065 microns.

anodic migration

separation time: 15 minutes

dyeing: toluidine blue O

3. DYEING OF GELS AND THICKENING MEANS ON CELLULOSE ACETATE FOILS

3.1 FIXING

Prior to dyeing, the gels and thickening means deposited on the cellulose acetate foils should be first fixed so as not to be dissolved during the dyeing process in the dyeing bath. As other egg white bodies, gelatin may be denatured by drying between 80 and 100°C and therefore be fixed, but this is not recommended for cellulose acetate foils, unfortunately, because of the wear on the foil. We make use of a fixing bath instead of the drying process, such bath consisting of a solution of trichloracetic acid for gelatin and of ethanol for the gels and thickening means of the polysaccharide group.

3.2 GELATIN DYEING

Dyeing of gelatin, which belongs to the protein group, occurs with the conventional egg white substance amido black 10 B, an acid azo-dye. The dyeing process is based on salt formation of the dye's acid groups with the free groups from the egg white. For gels and thickening means from the polysaccharide groups, dyeing must be undertaken in another manner, because there are no free amino groups and therefore do not respond to amido black 10 B (see fig. 7 and table 1).

3.3 PAS DYEING

The PAS (PERIODIC ACID SCHIFF) reaction, recommended by HOTCHKISS (1948) for the dyeing of polysaccharides in animal and plant tissue preparations used in histology, was carried over on our part to gels and thickening means. It was found that not all gels and thickening means could be dyed in this manner. Therefore we make a distinction between PAS positive and PAS negative gels and thickening means.

The Schiff reagency used in PAS dyeing consists of a colorless solution of fuchsin-sulfur acid prepared from adding potassium pyrosulfite and hydrochloric acid to a red, aqueous fuchsin solution. The released sulfur dioxide reduces the red fuchsin to a colorless leuko compound. This Schiff reagency is a known reagency test for aldehyde groups. The gels and thickening means to be dyed first are allowed to react with periodic acid for the generation of aldehyde groups. Two neighboring hydroxyl groups in the polysaccharide molecule will be oxidized to aldehyde groups in the presence of C-C fission. These aldehyde groups react with the Schiff reagency and generate a lilac -red color. Carubin, guaran, soluble starch, dextrin, alginate, tragacanth and gum arabic belong to the PAS positive gels and thickening means. Sodium pectate and carrageen are slightly PAS positive, carrageen's color being very slow to appear.

Polysaccharides, methyl cellulose, cellulose acetates and agar-agar, that lack neighboring hydroxyl groups, correspondingly are PAS negative. Water soluble methyl cellulose shows an average substitution index of 1.64 - 1.92 (GLICKSMAN, 1963) and also contains methyloxyl groups not only as regards the carbon atom C-6 but also to some extent the carbon atoms C-2 and C-3.

For negative PAS and slightly positive PAS gels and thickening means, we found two other ways of dyeing:

1. a prior treatment with tannin and subsequent PAS dyeing,
2. dyeing with toluidine blue O.

3.4 TANNIN TREATMENT AND PAS DYEING

We first considered using tannin for fixing the gels and thickening means. It was then shown that following such treatment, methyl cellulose could be dyed with the PAS reaction. Previously we had spent much time in vain looking for a way of dyeing methyl cellulose. Besides the latter, gelatin, carubin, agar-agar, tragacanth and gum arabic may also be rendered visible in this fashion. The tannin reaction occurring during this dyeing process -- if so -- is not known to us, except for the corrosive effect.

Besides tannin, we tested many other precipitation means for the polysaccharides, such as lead acetate, phosphor tungsten acid, mercuric chloride and barium chloride, though without satisfactory results obtaining. It was solely when pre-treating with barium chloride that better dyeing of carboxymethylcellulose was achieved. However toluidine blue O provides more sensitive dyeing.

Fig. 7
Dyeing of gels and thic kening means on cellulose acetate foils

Numbers 1 through 13 refer to the gels and thickening means in table 1 below. In this dyeing test, the samples were deposited by means of a stamp and about 1.5 cm apart on a membrane supported from a frame; membrane size was 2.5 x 30 cm. Each amount deposited contains about 6 ul of a 1% solution, that is, about 60 ugm of the particular gel or thickening means. Execution of dyeing is described in section 4.4.

3.5 DYEING WITH TOLUIDINE BLUE O

Toluidine blue O is a thiazine dye used in histochemistry for dyeing acid mucopolysaccharides such as heparin and chondroitin sulphates (RIENITS, 1953). Polysaccharide affinity for toluidine Blue O assumes the presence of acid groups in the molecule. Those gels and thickening means that may be dyed with toluidine blue O, that is, sodium pectate, alginate, tragacanth, gum arabic, carboxymethylcellulose and carrageen, contain either uronic acids or sulfate groups in the molecule.

3.6 RESULTS

By making use of the four different dyeing processes, all gels and thickening means admitted under article 443 bis of the Swiss Food Regulations may be dyed differently on cellulose acetate foils (fig. 7 and table 1).

Table 1
Affinity of gels and thickening means on cellulose acetate foils

Gelier- und Verdickungsmittel	Amido-schwarz 10B	PAS-Anfärbung	Tannin-behandlung + PAS-Anfärbung	Toluidin-Blau O.
1. Gelatine	[illegible]			
2. Na-Pektat	[illegible]	–	( )	-
3. Carubin* ××	–	( )	-	(+)
4. Guaran* ××	-	[illegible]	[illegible]	–
5. Lösliche Stärke				-
6. Dextrin			–	-
7. Agar-Agar	[illegible]		–	
8. Carrageen				–
9. Alginat			(+)	[illegible]
10. Tragant	[illegible]	[illegible]	–	
11. Gummi Arabicum		[illegible]		[illegible]
12. Methyl-Cellulose			[illegible]	-
13. Carboxy Methyl-Cellulose	-	( )		

++ high affinity
+ good affinity

(+) slight affinity
– no affinity

From the dyeing results for the gels and thickening means tested and listed in fig. 7, a listing is made in table 1 where the dyeing methods of the four different kinds used are referred to by plus or minus signs for easier visualisation.

4. PROCEDURE

4.1 SPECIAL EQUIPMENT

BECKMAN-SPINCO microzone electrophoresis system***, consisting of:

microelectrophoresis cell
micro-sample depositing stamp (0.25 ul sample)
cellulose acetate foil (also designated as membrane), 5.5 x 14 cm for 8 samples at a time
power supply (potential: 0-500 volts, 0-50 ma)

FOR ELECTROPHORESIS ON LARGE CELLULOSE ACETATE FOILS:

Elphor-H electrophoresis chamber of GRASSMANN & HANNIG, with associated rectifier and regulating transformer (Bender & Hobein, Munich, Zurich)
membranes of 4 x 30 cm (Schleicher & Schuell, AG, Feldmeilen)
sample depositing-stamp, about 6 ul sample (Kontron AG, Zurich)

REAGENTS:

borate buffer (pH=10, ion size = 0.13 microns): 12.37 gm (= 0.2 mol) boric acid in 100 ml 1-n NaOH solution, completed with distilled water to 1 liter. Then mix 600 ml of this solution with 400 ml of 0.1-m NaOH.

sodium carbonate, sodium hydrogen carbonate buffer (pH=10, ion size = 0.15 microns): mix 750 ml 1-m sodium carbonate solution with 750 ml of 0.1-m sodium hydrogen carbonate solution and with 500 ml distilled water.

PERIODIC ACID SOLUTION: 2 gm periodic acid in 10 ml distilled water solution, then mix with 90 ml 96% vol. ethanol.

TRICHLORACETIC ACID: 5% aqueous solution

TANNIN SOLUTION: 10% aqueous solution ****

SATURATED AMIDO BLACK 10 B SOLUTION: about 0.1 gm amido black in a mixture of 9 parts volume methanol and 1 part volume glacial acetic acid, dissolution through repeated shaking. Solution must be filtered prior to use.

SCHIFF's REAGENT: 1 gm fuchsin in 100 ml distilled water, hot dissolution, cooling to about 50°C; mixing with 1 ml concentrated hydrochloric acid and 2 gm potassium pyrosulfite, hard shaking, rest over night. Shaking with about 1gm charcoal prior to use and filtering.

TOLUIDIN O SOLUTION: 0.2% aqueous solution

formaldehyde-AMMONIA MIXTURE: mix 1-m formaldehide solution with 1-m amoonia hydroxide solution in equal parts volume.

ALCOHOL-HYDROCHLORIC ACID MIXTURE: mix 1 part volume 1-n hydrochloric acid with 2 parts volume denatured alcohol (aceton spirit).

METHANOL - GLACIAL ACETIC ACID MIXTURE: 1 part volume glacial acetic acid with 9 parts volume methanol

TRANSPARENCY SOLUTION: 1 part volume glacial acetic acid and 3 parts volume methanol (always fresh).

methanol-SOLUTIONS OF TESTED GELS AND THICKENING MEANS: 1% solution of individual gels and thickening means in the borate buffer. Exception: a concentration of only 0.3% for agar-agar, because a 1% solution may already cause gel binding. As regards carubin, guaran and tragacanth, only about 20% of the substance in borate buffer goes into solution.

4.3 EXECUTION OF MICRO-ELECTROPHORESIS

4.3.1 FILLING THE MICRO-ELECTROPHORESIS CELL WITH BUFFER SOLUTION

The micro-electrophoresis cell consists of the buffer containers, of the electrode chambers, of a support frame for tensioning and a membrane, of a cell-cover and a cell upper part with slits and grooves for the micro-depositing stamp when depositing samples. Upon removing the cell cover, the cell upper part and the support frame, the siphon located between the electrode chambers will be made horizontal by means of one finger and the cell will be filled through the siphon opening to a height between the lines FLUID LEVEL with buffer solution. When testing gels and thickening means of the polysaccharide group, borate buffer with a pH of 10 and ion size of 0.065 microns is used, and for the gelatin test, a buffer of sodium carbonate / sodium hydrogen carbonate with a pH of 10 and ion size of 0.075 microns. The buffer solutions described in section 4.2 to that end will be first diluted with distilled water in equal parts volume. Following filling of the micro-electrophoresis cell, buffer drops on the cell wall above the buffer level are carefully removed by means of filter paper in order to avoid secondary contacts of electrical current beyond the membrane.

4.3.2 EMPLACING THE MEMBRANE

The membrane first is slightly wetted with the non-diluted buffer being used (see section 4.2). In order to wet evenly, the membrane is placed flat on the surface of the buffer solution. After wetting, the foil is dipped into the buffer solution by means of tweezers. White air inclusion spots are thus avoided. The wet membrane then is removed and easily compressed between two sheets of thick filter paper.

Next the evenly wetted membrane is so tensioned in the supporting frame that all the pegs of the latter fit into the membrane's holes, and therefore the membrane will be held equally tautly everywhere. The supporting frame with tensioned membrane will be so inserted in the micro-electrophoresis cell that the reference hole of the foil will precisely coincide with the numeral 1 of the numbers marked on the cell upper part. This aids remembering the sequence of the deposited samples.

4.3.3 SAMPLE DEPOSITION

After emplacing the cell upper part, deposition of samples may begin. By means of a glass rod, a drop of the solution to be tested is put on a glass plate that is kept neat. The micro-sample depositing stamp by means of its platinum laminae touches the sample drop and the liquid film so created is deposited on the membrane, the platinum laminae remaining about two seconds in touch with the membrane. Prior to the next sample deposition, the platinum laminae of the micro stamp will be rinsed with distilled water and dried by means of careful dabbing with filter paper. Once all eight samples have been deposited, the cell cover is put into position and electrophoresis migration may begin.

4.3.4 ELECTROPHORESIS MIGRATION

The micro-electrophoresis cell is so connected to the power supply that the location of sample deposition is on the side of the cathode. Sample migration is toward the anode. Terminal potential is set to 200 volts. For the buffer concentration used, the current density does not exceed 1 ma/cm of foil width (= 5.5 ma), which is a good value for the migration and separation of the samples that were tested. Upon termination of the desired test duration, which may range from 15 to 30 minutes, the current is shut off, the electrophoresis cell is opened, the membrane is removed and then dyed. Dyeing takes place as described in section 4.4.

4.4 DYEING OF GELS AND THICKENING MEANS

The four different dyeing methods will be discussed in the sections below.

4.4.1 AMIDO BLACK 10 B DYEING

This method is used for rendering visible gelatin besides other egg white bodies. In order to fix the egg whites, the membranes will be immersed for 2-3 minutes in 5% trichloracetic acid solution. Then dyeing takes place for 10 minutes in the saturated amido black 10 B solution. Bleaching of that part of the membrane not carrying egg white takes place when the foil is immersed in a mixture of methanol and glacial acetic acid. The solution will be decanted after 2-3 minutes and replaced by a fresh one. Following threefold washing, white foils are obtained, on which gelatin spots and possibly others appear dyed deep-blue. The foil is rendered transparent in accordance with section 4.5.

4.4.2 PAS DYEING

This method is used to render visible carubin, guaran, soluble starch, dextrin, alginate, tragacanth and gum arabic. The membrane is inserted for 5 minutes in a periodic acid solution. This results in oxidation and also simultaneously in fixing by means of the alcohol of the periodic acid solution. Thereafter the membrane is immersed for 10 minutes in Schiff's reagent so that the individual spots of the tested gels and thickening means appear dyed lilac-reddish. In order to remove the excess Schiff reagent, the membrane is placed for one minute in a mixture of formaldehyde and ammonium, so that solution and membrane become intensely red because of the released fuchsin. In order to bleach that part of the foil free of gels and thickening means, the foil will be rinsed first with denatured alcohol and then several times with the mixture of alcohol and hydrochloric acid. The colortone of gels' and thickening means' spots changes from lilac-red to red-violet. The foil is rendered transparent in accordance with section 4.5

4.4.3 PRE-TREATMENT WITH TANNIN AND PAS-DYEING

This method is mostly used for rendering visible methyl celllulose and agar-agar. Other gels and thickening means that may also be dyed in this manner are gelatin, carubin, tragacanth and gum arabic. The membrane is placed for 7-8 minutes in the 10% tannin solution and then for 5 minutes in the periodic acid solution. Upon periodic acid treatment, foil and solution turn brown. The brown solution is decanted and replaced by new periodic acid. Dyeing proceeds as described in section 4.4.2

4.4.4 TOLUIDINE BLUE O DYEING

This method is mostly used for rendering visible carrageen, carboxymethylcellulose and sodium pectate. Other gels and thickening means that may be dyed with toluidine blue O are alginate, tragacanth and gum arabic. The membrane is placed for 10 minutes in the dye solution. Then the major part of the excess dye is removed by compressing the dyed foil between two sheets of filter paper. Following rinsing with ordinary tap water, the membrane is air dried.

4.5 RENDERING FOILS TRANSPARENT FOR PHOTOMETRIC EVALUATION

Following dyeing, the membrane is immersed for 2-3 minutes in methanol. Then it is placed on a glass plate or disc and subjected for 30 seconds to a fresh mixture of 3 parts volume methanol and 1 part volume glacial acetic acid. Glass plate and membrane are removed from the methanol / glacial acetic acid bath. Upon removing the excess liquid with a rubber sponge or filter paper, they are dried at 110°C for five minutes in an oven. The now transparent pherograms may be kept on the glass plates. They may be carefully removed from the glass plates for the purpose of photometric evaluation and they may be placed in glassines or the likes. The foils treated with toluidine blue O are not subjected to the methanol / glacial acetic acid bath because the spots of the tested gels and thickening means would dissolve, except for carrageen.

Gratitude is expressed to Herr Wenger, chemist from Bern, for verification of this work.

Pouponiere Nestle, Fondation Louis Dapples, Vevey (Switzerland)

Schweiz. Med. Wschr. 82(10): 256-258, 1952.

Research on the Mode of Action of Nestargel

by Chr. Rivier

In a preceding article [1] we gave the excellent results which we had obtained in the treatment of habitual regurgitations and vomiting in the infant with a powder extracted from the carob bean (Nestargel).

It seemed to us interesting to carry out some investigations in order to attempt to determine the manner of action of this powdee. We studied:

A. The effect of the Nestargel on the gastro-intestinal transit,
B. The effect of the saliva and the gastric juice on the viscosity of the Nestargel.

A. Effect of the Nestargel on the gastro-intestinal transit

1. Duration of the gastro-intestinal transit. In order to find out whether the thickening Nestle powder accelerated the gastro-intestinal transit we studied in the infant with normal stools the duration of the transit of a simple meal (pelargon orange, Nido, concentrated sugared milk) with the addition of a little carmine powder and then that of a similar meal containing 1% Nestargel.

The tests were carried out on 8 infants (sucklings). For each infant the transits were repeated two to three times both with Nestargel and without Nestargel (total transits: 31).

We took the average duration of the transits without Nestargel and the average duration of the transits with Nestargel of each infant and we obtained the following results:

1 Age mois	2 Lait	Sans Nestargel 3 h.	Avec Nestarge 4 h.
4	Nido	12	14
3	Nido	23	20
5	Pélargon	13	14
4	Pélargon	12	12½
3	Pélargon	16½	19
5	Pélargon	10	10½
2½	Pélargon	11½	19
5	Lait concentré sucré 5	13½	17
	Moyennes générales 6	14¾	14½

1= age in months; 2= milk; 3= without Nestargel hours; 4= with Nestargel, hours

5= concentrated sugared milk; 6= overall averages

The above table shows that the addition of Nestargel does not change the duration of the gastro-intestinal transit, since the small differences found in one direction or the other fall within the margin of the normal variations.

2. Duration of gastric transit.

We also sought to find out whether there was a difference between the duration of the gastric evacuation after a meal without Nestargel and that of a meal containing Nestargel and we carried out 14 baryta transits at the Hopital du Samaritain hospital.

Four infants 2, 3, 4 and 6 months old were examined microscopically. The customary meal (Pelargon or Nido) along with 3 soup spoons of baryun was given with or without Nestargel (1%). Each gastric transit was examined from hour to hour.

We were able to note that the presence of Nestargel did not slow down in any way the evacuation of the gastric content. In the two cases the stomachs were always emptied after 3½ hours.

3. Aerophagia. The air bubble is clearly smaller after a meal containing Nestargelthan after a meal not containing Nestargel. It was possible to observe this in all of the infants in variable proportions. As an average, the air bubble forming after a meal without Nestargel occupied 33% of the stomach, whereas after the meal with Nestargel it was only 21%. Regardless of its size the air bubble was rapidly expelled

4. Consistency. The difference in consistency in the stomach between that with the thickening powder and that without powder is quite visible in radioscopy. When the infant is shaked after a meal without Nestargel the gastric liquid is seen to squirt out just as water in a bottle. In contrast, the meal containing Nestargel behaves just like a cream and squirts out with greater difficulty. This shows that the thick consistency of the meal continues in the stomach.

Conclusions:

The duration of the gastro-intestinal transit is not changed by the presence of Nestargel.

The addition of Nestargel to the meal does not change the duration of the gastric transit.

The physiological aerophagia is clearly less marked when Nestargel is added to the meal.

The consistency of the meal containing Nestargel changes very little in the stomach, in contrast to the starchy meals which are liquefied.

B. The effect of the saliva and the gastric juice on the viscosity of Nestargel

We studied the action of the digestive juices on a meal thickened with Nestargel and, by way of comparison, on a meal thickened with starch (Soldor). These tests were made in vivo and in vitro.

Method used to determine the viscosities. The method consists in allowing the liquid being examined to flow along a glass tube and to time the time of drop between two reference points. We used a glass tube with a diameter of 0.5 cm and placed the reference points at a distance of 35 cm. The tube was kept in a vertical position by means of a tripod.

The viscosities are established at 37°. The dropping times are expressed in seconds. The dropping time of the water is practically equal to 0. A liquid can be considered to be viscous with a dropping time greater than 2 seconds (it does not squirt out when we shake the recipient which contains it).

1. Examination of the viscosity of the meal in the stomach. Four infants aged 4 months received a feeding bottle of Eledon at 10% along with 1% of Nestargel.

1 Repas		2 Contenus gastriques	
Composition	3 Temps de chute sec.	4 Temps de chute sec.	pH
1. Eledon 10% + Nestargel 1%	35	9	4.5
Eledon 10% + Soldor 6%	214	0	4.7
2. Eledon 10% + Nestargel 1%	45	6	4.3
Eledon 10% + Soldor 6%	175	0	4.5
3. Eledon 10% + Nestargel 1%	59	6	4.1
Eledon 10% + Soldor 6%	[illegible]	0	4.0
4. Eledon 10% + Nestargel 1%	67	6	4.1
Eledon 10% + Soldor 6%	[illegible]	0	4.1

1= Meal; 2= gastric contents; 3. dropping time in seconds
4= dropping time in seconds

Twenty minutes after the end of the meal the gastric content is withdrawn. We compare the viscosity of the meal given to that of the gastric content.

The same experiment was then carried out with Eledon at 10% along with starch (Soldon at 6%). This meal has the consistency of a porridge, its viscosity is clearly greater to that of the meal with Nestargel.

The same investigations yielded the following results made:

a. with a meal of water and Nostargel or Soldor added
b. with a meal of Eledon with Nestargel or Soldor added

Meal		Gastric Contents	
Composition	dropping time sec.	dropping time sec.	pH
water + Nestargel 1%	45	5	3
water + Soldor 6%	249	0	3.3
reconstituted Eledon + Nestargel	178	57	4.2
reconstituted Eledon + Soldor	175	0	4.5

After meal with Nestargel the probing is long and difficult, the gastric content had difficulty in passing by the probe and it was necessary to suck it up with a syringe, which has the drawback of increasing the proportion of gastric juice and teducing the viscosity by dilution. The infant attempted to vomit but could not. The liquid which was obtained was thick, often ropy, sticking to the glass. It does not squirt out when shaken.

After meal with soldor the gastric content flows rapidly through the probe. The liquid is curdled, very fluid, it squirts out upon the slightest shaking. We noted that the meal with Soldor already started to liquefy in the mouth of the

infants.

Conclusions:

The meals with Nostargel are still viscous after remaining 20 to 30 minutes in the stomach , whereas the meals with Soldor did not present any viscosity.

2. Examination in vitro of the viscosity of the meal with artificial gastric juice added

Parallel to the tests in vivo, we made tests in vitro adding artificial gastric juice and saliva to mixtures of Eledon-Nestargel and Eledon-Soldor or to aqueous solutions of Nestargel and Soldor.

Artificial gastric juice. After several tests we used gastric juice at pH of 3.6. Formula: pepsin 2.5 g, HCl N 0.7 cm^3 , distilled water 100. After addition of this juice to the meal we added a bit of chlorhydric acid up to about pH 4.7 (minimum pH after a meal of milk cut at 1/3rd, according to Dr. L. Exchaquet, "The infant, its physiology and its health") . We placed 25 cm^3 of gastric juice per 100 cm^3 of the mixtures of Nestargel or Soldor and 15 to 20% saliva (Exchaquet).

a. Comparison between the aqueous solutions of Nestargel at 1% and of Soldor at 6.8%

Results

50 cm^3 of Nestargel at 1%		50 cm of Soldor at 6.8%	
pH	Dropping time	pH	dropping time
7	34"	7	40.6"

We add 12.5 cm^3 of gastric juice and 8 cm^3 of saliva + some drops of HCl N.

4.5-4.7	8"	4.5	

The mixture is kept at 37° C dry

½ hour later	8.4"
1 hour later	9.6"
1 hour 40 min.	10.0"
2 hours later	10.2"
2½ hours later	11"

b. Comparison between the mixture Eledon 10% + Nestargel 1% and the mixture eledon 10% + Soldor 6.8%

Results

100 cm^3 of Eledon+ Nestargel 1%		100 cm^3 of Eledon + 6.8% Soldor	
pH	dropping time	pH	dropping time
4.9	67"	4.9	32"

We add 25 cm^3 of pure gastric juice, 20 cm^3 of pure saliva, some drops of HCl N.

4.7	12"	4.7	0- 1/10"

The mixture is kept at 37° dry

¼ hour later	11"
½ hour later	11.4"

Conclusions. In vitro the viscosity of a solution of Nestargel in water or in Eledon is still very marked after 2½ hours of stay in the artificial gastric juice and saliva. The viscosity experiences an immediate drop after the addition of the digestive juices (probably by dilution) and increases gradually (probably by evaporation).

The viscosity of a solution of Soldor in water or in Eledon is immediately destroyed by the addition of the gastric juice and saliva.

We then attempted to determine the factors which reduced the viscosity of the Nestargel in the stomach and completely suppress that of the Soldor. Thus we studied:

1. the effect of the pH and of the pepsin
2. the effects of the dilution
3. the action of the saliva.

1. Effect of the pH and of the pepsin. To the aqueous solutions of Nestargel 1% and Soldor 6.8% we added directly pepsin in powder and HCl N in order to prevent dilution. The acid was added little by little up to pH 4.7.

Results

Nestargel 1%		Soldor 6.8%	
Dropping time	pH	Dropping time	pH
43"	7	45.8"	7

We add chlorhydric acid and pepsin

48"	4.7	40"	4.7

After 35 minutes in the acid medium (at 37°)

63"	4.7	34.8"	4.7

Neither the pH of the gastric juice of the infant, nor the pepsin reduced the viscosity of the Nestargel. This viscosity even seemed to have increased with the acidification of the medium (other factors, however, could be involved such as evaporation, thickening caused by the pepsin powder).

The viscosity of the Soldor dropped slightly.

2. Effects of the dilution. Comparison between the effect of the gastric juice and that of ordinary water on the viscosity.

Results

Test with the gastric juice		Test with ordinary water	
Dropping time	pH	Dropping time	pH
100 cm^3 of Nestargel 1%			
40.2"	7	43"	7
We add 25 cm^3 of gastric juice + several drops of HCl N.		We add 25 cm^3 of ordinary water	
17"	4.7	19.5"	7
100 cm^3 of Soldor			
46.6"	7	41.8"	7
We add 25 cm^3 of gastric juice + several drops of HCl N.		We add 25 cm^3 of ordinary water	
11"	4.7	7"	7

Ordinary water added in the same quantities as the gastric juice reduces the viscosity of the Nestargel and of the Soldor in the same proportions.

3. Action of the saliva (ptyaline). a. Action on the Nestargel: 20% of the infant's saliva placed in a solution of Nestargel 1% only causes a very slight drop in its viscosity (probably the effect of dilution).
b. Action on the Soldor: 15% of adult or infant saliva placed in a 6% solution of Soldor immediately liquefies it.

The ptyaline liquefies the Soldor. It does not have any effect on the Nestargel.

Conclusions:

Neither the ptyaline nor the pepsin nor the pH of the gastric juice of the infant are capable of reducing the viscosity of the Nestargel. The reduction in the viscosity of the Nestargel in the gastric juice seems to be due solely to the dilution by the digestive juices.

The liquefaction of the Soldor can be explained entirely by the action of the ptyaline, the dilution by the digestice juices only playing a secondary part.

Summary. The duration of the gastro-intestinal transit is not changed by the presence of Nestargel in the meal if the infant's digestion is normal.

The duration of the gastric transit is not changed by the presence of Nestargel.

The aerophagia is definitely weaker when the meal contains Nestargel.

The meal thickened with Nestargel retains a strong viscosity during the entire gastric digestion.

The viscosity of the meal thickened by Nestargel is not changed either by the action of the saliva or by the digestive action of the gastric juice. It is slightly reduced by the dilution due to the digestive juices, whereas the starch porridge is rapidly liquefied by the action of the saliva.

General conclusion:

The addition of Nestargel to the meal causes a reduction in the physiological aerophagia.

Since the Nestargel is not changed by the salive and the gastric juice, the meal retains a thick consistency during its passage into the esophagus and stomach.

These two factors probably play an important role in the very favorable action of the Nestargel on the habitual regurgitations and vomitings of the infant.

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Growth Inhibitory Effect of Certain Polysaccharides for Chickens

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IT IS well-known that the growth of chickens is inhibited if their diet contains either linseed meal (Kratzer and Williams, 1948; MacGregor and McGinnis, 1948), or ground carobs (Kratzer and Williams, 1951; Bornstein *et al.*, 1963), or guar meal (Borcher and Ackerson, 1950; Vohra and Kratzer, 1964). In each case, the feces of the chickens were extremely sticky and tended to paste up the vent. Linseed meal loses its growth-inhibitory properties if it is given a preliminary water treatment; or if the pyridoxine content of the diet is increased (Kratzer and Williams, 1948; MacGregor and McGinnis, 1948). Similar treatment of ground carobs

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are either very slight but significant (Kratzer and Williams, 1951), or no growth improvement (Bornstein *et al.*, 1963) of chicks. Our unpublished studies indicate that guar meal is not improved either by treatment with water or by the addition of pyridoxine.

Linseed meal, carob and guar seeds are excellent sources of polysaccharides which are classified as gums and mucilages. An obvious question in considering these materials is whether this growth depressing effect is due to the high levels of polysaccharides which they contain.

A part of the growth inhibitory properties of linseed meal has been attributed to its mucilage content (Mani *et al.*, 1949; Clagett *et al.*, 1955) even though the latter workers doubted this in another report (Schlamb *et al.*, 1955). A depression in the growth of rats has been observed when their diets contained more than 5% agar agar or Irish moss (Nilson and Schaller, 1941). The digestibility of agar agar, pectin and guar gum is reported to be 21%, 19% and 76% respectively, and no significant depression in the growth of rats was observed when the diets contained these polysaccharides at levels of 15%, 10% and 6%, respectively (Booth *et al.*, 1963). Ershoff and Wells (1962) found no significant depression in the growth of rats which were fed 10% pectin, guar gum, locust bean gum or carrageenan. Carrageenin appears to interfere with the digestion of proteins by pepsin under certain conditions (Vaughan *et al.*, 1962). The present work which was reported in abstract form (Kratzer and Vohra, 1963) is a study of the effects of feeding polysaccharides on the growth of chickens.

EXPERIMENTAL

Commercially prepared samples of the following polysaccharides were used in this study, guar gum,[1] locust (or carob) gum,[1] gum arabic,[1] gum ghatti, gum tragacanth,[1] gum karaya, pectin, carrageenin, cellulose,[3] methylcellulose, sodium salt of carboxymethylcellulose, agar agar, dextrin, dextran and polygalacturonic acid.[2]

Dried okra powder was prepared by homogenizing fresh okra in a blender and an excess of methanol was added to the homogenate. The mixture was stirred and filtered on a Büchner funnel under vacuum. The solid residue was dried in a current of air at room temperature, and ground.

To obtain mucilage, linseed oil meal was soaked in an excess of water overnight, stirred and filtered through muslin. The volume was reduced by leaving the mucilage in shallow pans in a draft of air. The polysaccharide was precipitated from the mucilage solution by the addition of methanol. It was filtered and dried in a current of air at room temperature.

Caramel was prepared by the heating of sugar crystals (sucrose) in an iron crucible over a burner.

Psyllium husk and dried kelp were obtained from commercial sources.

The polysaccharides were added to the stock diet (Table 1) at the required level without making any adjustment in its composition (Table 1) at the required level without making any adjustment in its composition. Day-old, Arbor-Acres broiler type chicks were weighed and distributed into groups of approximately equal average weight. They were housed in electrically heated batteries in which water and feed were available *ad libitum*. The chicks were weighed twice every week as groups and individually at the termination of the experiment.

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[1] Samples through courtesy of Stein, Hall and Co., Inc.

[2] Through courtesy of Dr. M. A. Joslyn.

[3] Solka-floc, Brown Co., Berlin, New Hampshire.

TABLE 1.—*Composition of the stock diet*

Ingredient	Inclusion, gm./kg.
Ground milo	175
Ground corn	157
Ground barley	200
Bran, wheat	150
Soybean meal, 44% protein	120
Fish meal	75
Meat and bone meal, 50% protein	12
Whey, dried	25
Milk, skimmed, dried, 35% protein	25
Alfalfa meal	40
Limestone, ground	15
Salt*	5
Vitamins†	1

* Contains 0.025 gm. manganese sulfate (70% feed grade).

† Supplies: riboflavin, 1.1 mg.; niacin, 1.1 mg.; Ca-pantothenate, 1.1 mg.; choline chloride, 5.5 mg.; sulfaquinoxaline, 125 mg.; vitamin A, dry (20,000 I.U./gm.), 375 mg.; vitamin D_3, dry (4,500 I.C.U./gm.), 250 mg.; and folic acid, 33.3 μg.; and bran to make 1 gm.

A slurry of 120 gm. guar gum in 1500 ml. of water was reacted overnight at 37°C. with 100 ml. of a solution containing 6 gm. of either Cellulase-100[4], or the isolated enzyme from the sprouted guar beans (Vohra and Kratzer, 1964). The mucilage broke down in this process and a fluid was obtained which was reduced to about half its volume in shallow pans in a current of air at room temperature. This concentrated solution was mixed with 5880 gm. of stock diet and the diet was fed as such without any further drying.

[4] Through courtesy of Miles Chemical Co.

Pectin (240 gm.) was stirred in 2 liters of water to give a thick, viscous mass which was reacted with 4.8 gm. of pectinase in 100 ml. water at 37°C. for about 12 hours. The viscous mass was stirred frequently and yielded a fluid solution which was concentrated to about 800 ml. in shallow pans in a draft of air and mixed wtih 5760 gm. stock diet to feed the chicks. It was not dried further.

The relative growth value compares the final average weight of the chickens fed the experimental diet with the average weight of chickens fed the stock (control) diet in the same experiment. For the comparison, the relative weight of the group fed the control diet is adjusted to 100.

TABLE 2.—*Relative growth (R.G.) and gm. feed/gm. gain (F/G) of chickens fed various polysaccharides in their diets*

Experiment Series	1	2	3	4	5	
Duration of trial, days	20	21	20	21	20	
Polysaccharide and its level	R. G.	R. G.	R. G.	R. G.	R. G.	F/G
Control	100	100	100	100	100	1.73
Cellulose, 2%	101	92				
Guar gum, 2%	67.4	64		61		
Guar gum, 1%	84.4	80		72.2	79.1	1.70
Guar gum, 0.5%				78.6	92	[illegible]
Guar gum, 0.25%				89.1	98.4	[illegible]
Locust gum, 2%	73	73.6				
Locust gum, 1%			96.8			
Locust gum, 0.5%			87.8			
Locust gum, 0.25%			96.5			
Gum tragacanth, 2%	65.8	66				
Gum arabic, 2%	96.5	85.6				
Dextran, 2%	94.8					
Dextrin 2%		103				
Pectin, 4%	88.6	77.5		89.5		
Pectin, 2%	92.4	86.5				
Kelp, 4%			99.5			
Kelp, 2%			99.5			
Actual gain of control, gm.	256	330	332	294	297	

TABLE 3.—*Relative growth (R.G.) and mg. pancreas/100 gm. body weight (P/B.W.), gm. feed/gm. gain (F/G), of chickens fed various polysaccharides in their diets*

Experiment Series	6		7		
Duration of trial, days	20		20		
Polysaccharide, and its level	R. G.	P/B.W.	R. G.	P/B.W.	F/G
Control	100	375	100	342	1.54
Cellulose, 2%	96.8				
Okra, dried, 2%	87.6	470	81.8	462	1.84
Carrageenin, 2%	75	532	75.4	555	1.91
Linseed mucilage, 2%	95.4	370	97.4	—	1.84
Psyllium husk, 2%	87.4	435	81.8	456	1.92
Methylcellulose, 2%	103				
Carboxymethylcellulose, (Na), 2%	96.5	360	95.8	—	1.88
Caramel, 2%	100				
Actual gain, gm.	339		320		
Control			100	474	1.78
Kelp, 2%			114	506	1.59
Agar agar, 2%			110	415	1.72
Gum karaya, 2%			72	432	1.67
Gum ghatti, 2%			104.5	427	1.81
Actual gain, gm.			280		
Control			100	410	1.78
Gum ghatti, 2%			95		1.81
Gum karaya, 2%			70	500	1.92
Agar agar, 2%			102		1.87
Actual gain, gm.			315		

RESULTS AND DISCUSSION

The relative growth of the chickens fed the diet diluted with 2% of cellulose was 92 to 101% of the controls (Table 2 and 3). When the diets contain 2% guar gum, the relative growth of the chickens was 51 to 67.4% of the controls. The depression in growth was probably not due to the dilution of the energy content of the diet by the addition of 2% gum because the addition of 2% cellulose which also diluted the energy of the diet to the same extent, caused little or no growth depression. The addition of the following polysaccharides at a level of 2% to the chicken diets caused a definite depression in growth of chickens: locust gum, gum tragacanth, gum karaya, dried okra, carrageenin, and psyllium husk (Tables 2 and 3). Pectin exhibited a marked depression in chick growth at a level of 4%. At a level of 2%, growth of chickens on diets containing pectin, gum arabic, dextran, dextrin, gum ghatti, linseed mucilage, methylcellulose, carboxymethylcellulose, caramel, agar agar or kelp was of the same order as for control diets or those containing 2% cellulose.

As low as 1% guar gum in chicken diets caused a definite growth depression (Table 2). As the level of guar gum was decreased to 0.5 or 0.25%, the growth inhibition was also proportionately reduced. However, in case of locust gum, a definite growth inhibition of chicks occurred only at a 2% level.

Relation to composition. The variation in growth depressing effect of various polysaccharides prompted consideration of a possible common constituent responsible for this effect. Most of the data on the chemical constitution of these polysaccharides have been taken from the reviews of Smith and Montgomery (1959).

Guar gum and locust gum are neutral

polysaccharides containing 33-36%, and 14-25% D-galactose; and 64-67% and 75-86% D-mannose, respectively. Guar gum was far more growth inhibitory than locust gum at a level of 1% of the diet. D-galacturonic acid content of gum karaya and okra mucilage is 43% and 6-8%. It is also present in gum tragacanth, linseed mucilage, psyllium husk and pectin but the exact amounts are not known. D-glucuronic acid is present to an extent of 16% in gum arabic; and 12% in gum ghatti. D-galactose content of the gums is as follows: arabic, 52%; ghatti, 27%; karaya, 14%; okra mucilage, 79-80%. Linseed mucilage contains L-galactose. D-galactose is also present in gum tragacanth, agar agar and carrageenin. About 8% D-mannose is present in gum ghatti. Gum tragacanth and psyllium contain L-arabinose which is estimated to be 19% in gum arabic, 41% in gum ghatti, 3-14% in okra mucilage and 12% in linseed mucilage. L-rhamnose is present in gum arabic and its content in gum karaya and linseed mucilage is 15% and 29%, respectively. However, no prediction is as yet possible about the growth inhibitory properties of polysaccharides from their chemical constitution.

The growth inhibition is probably not due to the mucilage character of the polysaccharides in general because not all of them have exhibited growth inhibitory properties for chickens in this study. If those polysaccharides which do exhibit growth depression are allowed to be reacted with suitable enzymes, the reaction products cause no depression in the growth of chickens (Table 4). Guar gum (2%) when fed along with an enzyme capable of hydrolyzing it, at a level of 0.1%, overcame the growth inhibition of chickens to some extent. However, the growth of chickens fed guar gum which has been hydrolyzed with the enzyme was of the same order as on control diets. The enzyme was either isolated from the sprouted guar beans or a commercial preparation, Cellulase-100, capable of splitting polysaccharides. Growth inhibition due to 4% pectin was completely overcome if it was first re-

TABLE 4.—*Effect of enzymes on the relative growth (R.G.), mg. pancreas/100 gm. body weight (P/B.W.), and gm. feed/gm. gain (F/G) of chickens fed various polysaccharides*

Experiment Series	8			9		
Treatment and level	R. G.	P/B.W.	F/G	R. G.	P/B.W.	F/G
Control	100	474	1.78	100	410	1.78
Guar gum, 2%	69.4	510	1.58	61.6	620	2.35
Guar gm. 2%+0.1% guar enzyme	79	508	2.17	76.5	474	2.32
Control+0.1% guar enzyme	117	376	1.63	102	—	1.86
Guar gum (2%) reacted with guar enzyme	102.5	386	1.60	95	378	2.[illegible]
Control+0.1% Cellulase-100				100		1.9[illegible]
Guar gum 2%+Cellulase-100, 0.1%				86	356	2.02
Actual gain, gm. (Experimental period)	280 (20 days)			315 (20 days)		
Control	100			100		
Pectin, 4%	61.5			69.2		1.91
Pectin, 4%+0.08% pectinase	84			83		1.97
Pectin, (4%) reacted with pectinase	100			102		1.[illegible]
Control+0.08% pectinase	95.5			98.8		1.75
Actual gain, gm. (Experimental period)	294 (21 days)			297 (20 days)		
Control	100					
Pectin, 4%	53.5					
Polygalacturonic acid, 4%	89.8					
Actual gain, gm. (Experimental period)	327 (20 days)					

ated with the enzyme pectinase. When pectin and the enzyme were fed together, the growth inhibition was only partially overcome. The presence of methoxy groups in pectin was essential for its growth inhibition. This is borne out by the fact that polygalacturonic acid when fed at a level of 4% gave a relative growth of about 93% of the controls and the methoxy derivative of this (pectin) had a value of about 54%.

No information, as yet, is available about the exact sequence in which the various sugars are linked in the various polysaccharides. But it appears that as the frequency of branching increases in the molecule, the growth inhibitory properties also increase. Guar gum has D-galactose on every alternate D-mannose molecule in contrast to carob gum which has D-galactose on every 3rd or 4th mannose molecule of the straight chain part of the polysaccharide. Guar gum is more growth inhibitory than locust (carob) gum. Gum arabic has fewer branches and more of the component sugars are arranged as a straight chain. It has little growth inhibitory properties for chicks. In contrast to this, gum tragacanth has many more sugars branching from the straight chain of the polysaccharide and has definite growth inhibitory properties.

The ratio of feed intake/gain for controls and the diet containing 2% guar gum was of the same order (Table 2), but tended to be higher for okra, carrageenin, linseed mucilage, psyllium husk and carboxymethycellulose (Table 3). Not all of these substances were growth inhibitory.

In general, the pancreas weight per 100 gm. body weight (Table 4) tended to be higher for those polysaccharides which had a growth inhibitory property. Chickens fed psyllium husk had an accumulation of mucilage in the proventriculous which gave it an abnormal appearance of a sausage similar to ones observed by O'Dell *et al.* (1959). The incidence of this gross abnormality was only in chickens fed growth-inhibitory polysaccharides but was never as serious as for psyllium husk.

SUMMARY

Several naturally occurring polysaccharides depressed the growth of chickens when fed in a nutritionally balanced diet containing soybean meal, cereals, and fish meal. The growth of chickens was inhibited about 25% to 30% by the inclusion of guar gum, locust gum, gum tragacanth, gum karaya, or carrageenin at levels of 2%; or 4% of pectin in their diets. Dried okra or psyllium husk caused a depression in growth of about 15% at a level of 2% inclusion, and cellulose, methylcellulose, carboxymethylcellulose, dextrin, dextran, linseed mucilage, caramel, gum ghatti, agar agar and kelp appeared to be without any deleterious properties. The growth depressing properties of pectin and guar gum were overcome by their treatment with enzymes capable of hydrolyzing them, namely, pectinase and cellulase or a preparation from the sprouted guar beans.

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